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Lellingeria cantarensis (Polypodiaceae): A New Dwarf Species from Cerro Jefe, Panama

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Abstract.—A new species, *Lellingeria cantarensis,* is described from the cloud forests of Cerro Jefe, an isolated mountain peak in central Panama known for its high levels of endemism and species diversity. It appears most similar to *L. kaieteura* and *L. epiphytica*, both of which also have dorsiventral rhizomes, clathrate rhizome scales with ciliate margins and abaxial hairs on the rachis. The new species is distinct from those two species, and from all other congeners, by its small laminae (less than 6 cm long), truncate laminar base, and the abaxial side of rachis is densely pubescent. *Lellingeria subimpressa* is also similar to *L. cantarensis* but it differs by having a glabrous rachis. None of the similar species are sympatric with *L. cantarensis*; they are restricted to Costa Rica, South America, or Caribbean Islands. A phylogenetic analysis using five plastid markers and 94 other grammitid taxa confirms the new species as sister to the *Lellingeria suprasculpta* clade with strong support. *Lellingeria cantarensis* is known only from the type and one additional collection from the type locality, and it is one of ten *Lellingeria* species found in Panama; it is also the only endemic *Lellingeria* in the country. We also provide a key to the species that occur in Panama and neighboring Costa Rica. With the addition of this new species, the total number of *Lellingeria* species becomes 51 worldwide.

Key Words.—endemic species, epiphyte, grammitid fern, Mesoamerica, phylogenetic analysis

The Cerro Jefe is the tallest peak on the western flank of the San Blas Mountains northeast of Panama City, Panama (Fig. 1). This area is characterized by windswept cloud forests between 800 m and 1000 m above sea level (ANAM, 2000) and an average annual rainfall of 2050 mm (ETESA, 2008). Cerro Jefe is also known for high levels of plant diversity and endemism. It is home to a broad array of Neotropical flowering trees and shrubs, with a high number of epiphytic vascular plants and bryophytes. Of the approximately 1,016 reported plant species, 66 are exclusive to Cerro Jefe (De Sedas *et*

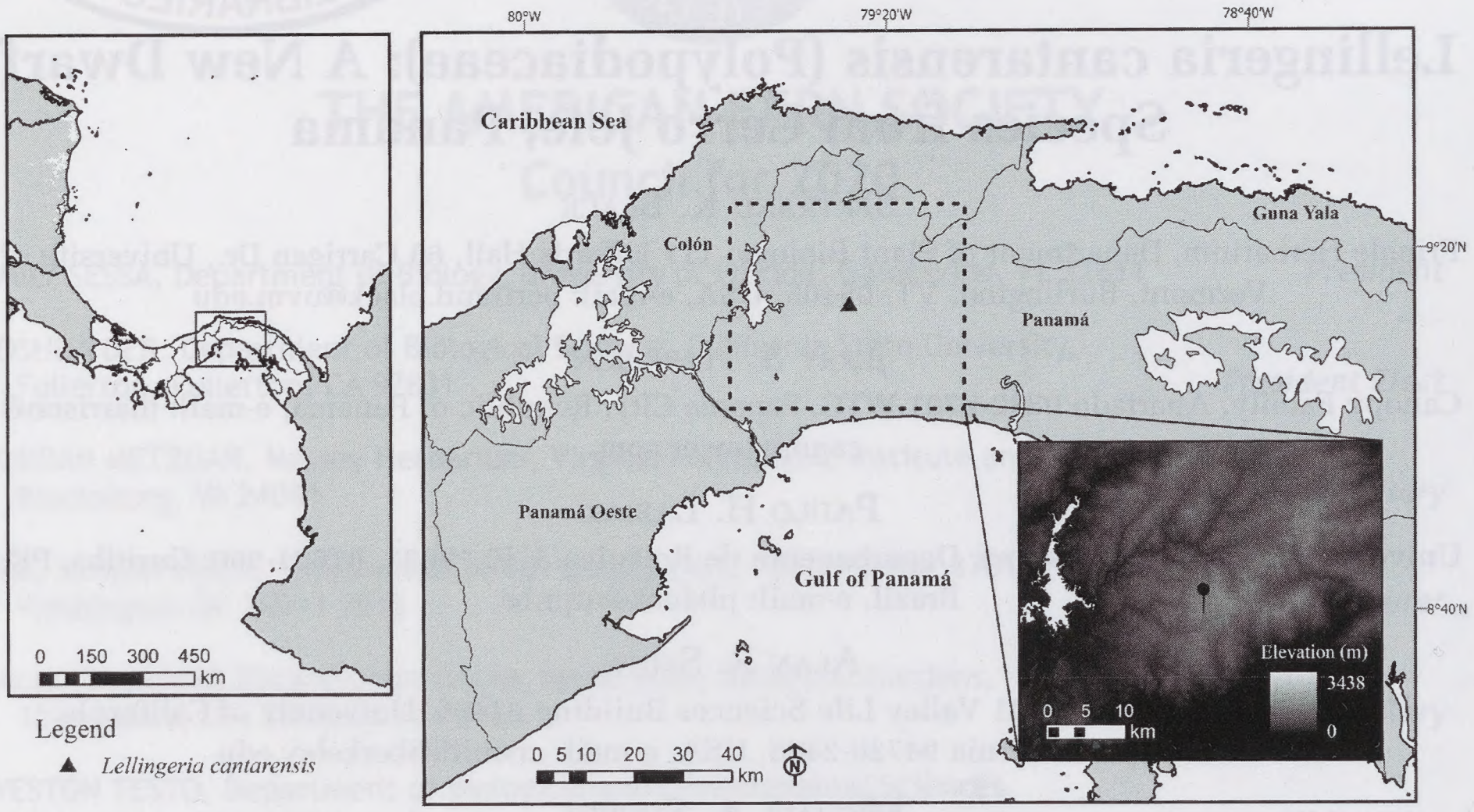

FIG. 1. Distribution of *Lellingeria cantarensis* in Panama. Map made on ArcGIS Pro, version 2.5.0 (https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview).

al., 2009), and new endemic species continue to be described from this locality (Almeda, Marcela, and Mendoza, 2014; Berry and Galdames, 2013; Kuijt and Harrison, 2015). De Sedas *et al.* (2009) suggested that the high levels of endemism found on Cerro Jefe today may be attributed to its rich history of geographic and climatic isolation. The highlands that are now Cerro Jefe were formed as a volcanic archipelago known as the Panama–Costa Rica Arc during the early Paleocene to late Oligocene (66–23 Mya) and were some of the first landforms to emerge in the Central American Seaway that existed between North and South America (Harmon, 2005; Kesler *et al.*, 1977; Maury *et al.*, 1995). This pre-isthmian archipelago permitted some plants to island hop between the two continents and may have contained their own endemic floras, such as those found in the West Indies today (Dick, Condit, and Bermingham, 2005).

On the foothills of Cerro Jefe, near the subdivision of Los Altos de Cerro Azul, lies the Cantar Trail. This trail passes through protected forest as it follows the small Río Vistamares SSW for several kilometers, ranging in elevation from 700 m to 900 m. The area has seen little development and remained relatively inaccessible until the creation of the Cantar trail in the mid-1980s. As a result, the habitat along this trail is an important refuge for the species of Cerro Jefe and is the type locality for many of the newly described endemic species, such as *Dendrophthora fortis* Kuijt, *Croton cerroazulensis* P.E. Berry & Galdames, and *Graffenrieda jefensis* Almeda, Alvear & Humberto Mend. While conducting floristic surveys along the Cantar Trail, Jerry and Linda Harrison encountered a dwarf species of epiphytic fern (Fig. 2). It appeared most similar to *Lellingeria kaieteura* (Jenman) Labiak and was

FIG. 2. *Lellingeria cantarensis*, habit, *J. Harrison and L. Harrison 865* (VT).

hypothesized to be a close relative, although *L. kaieteura* is a species not known to occur in Panama and known only in Colombia, Cuba, Trinidad and Tobago, and Venezuela (Labiak, 2013).

Lellingeria A.R.Sm. & R.C.Moran is a Neotropical genus of 50 species in the Polypodiaceae subfamily Grammitidoideae Parris & Sundue (Labiak, 2013; PPG I, 2016; Smith, Moran, and Bishop, 1991; Sundue *et al.*, 2014; Sundue, 2019). The subfam. Grammitidoideae can be distinguished from other taxa in the Polypodiaceae by having green chlorophyllous trilete spores, laminae with setae that lack scales, and sporangial stalks that are one cell wide in the middle as opposed to three cells wide as seen in most Polypodiales (Parris, 1990; Sundue, 2010; Sundue, Islam, and Ranker, 2010). *Lellingeria* can be diagnosed by its dark clathrate rhizome scales, hydathodes, the loss of the red setae, and by its characteristic branched hairs. These hairs are asymmetrically 1–2-furcate, and usually comprise a pale basal cell with 1–2 acicular cells attached by smaller undifferentiated cells (Labiak, Sundue, and Rouhan, 2010). In Panama, there are ten known *Lellingeria* species, of which six are endemic to the Central American region (Labiak, 2013). We here report the discovery of a new species known only from the type location of Cerro Jefe; it is the first endemic Panamanian *Lellingeria*.

Materials and Methods

Molecular Phylogenetic Analyses.—The systematic position of the new species was assessed using 29 other species of *Lellingeria* for which sequence data were available from GenBank; the sampling also included 65 grammitid outgroups, representing eight genera. We extracted DNA from both the type (*J. Harrison & L. Harrison 865*) and an additional collection (*J. Harrison & L. Harrison 821*). Five plastid DNA markers were PCR-amplified from these two accessions, including the *trnG-trnR*, *trnL-trnF*, and *rps4-trnS* introns, as well as the *rbcL* and *atpß* coding regions. Protocols and primers sets from Sundue *et al.* (2014) were followed for DNA extraction and PCR amplification. All markers were recovered from *J. Harrison & L. Harrison 865*, and two markers were recovered from *J. Harrison & L. Harrison 821*. DNA sequencing was performed by the Vermont Integrative Genomics Resource in Burlington, Vermont. Sequence data generated for *L. cantarensis* were submitted to GenBank. Accession numbers and vouchers for all sequences used in our analyses are provided in Appendix 1.

Sequences were edited and contigs were produced using Geneious 6.17 (Biomatters Ltd., San Francisco, California), and the MAFFT plug-in was used to produce alignments (Katoh and Standley, 2013). Phylogenetic trees were constructed using two methods: Maximum Likelihood (ML) and Bayesian Inference (BI). They were implemented by using RAxML-HPC2 (Stamatakis, 2006) and MrBayes 3.2.7a (Ronquist *et al.*, 2012) through the CIPRES portal (Miller, Pfeiffer, and Schwartz, 2011). In RAxML, trees were generated using a GTR + CAT model for nucleotide substitution, 1000 rapid bootstrap (BS)

replicates, and investigated for the best-scoring ML tree. In MrBayes, two independent analyses were performed in parallel; of the four chains one was cold and three incrementally heated at a temperature of 0.2. The analyses were each run for 10 million generations each and sampled every 1000 generations (10,000 total trees). Tracer 1.7.0 (Rambaut *et al.*, 2018) was used to estimate convergence and control for effective sample sizes. For both independent analyses, sample sizes were shown to be above 200 and to have achieved convergence to the stationary distribution. Burn-in was set at 25% and the remaining trees were pooled, and a 50% majority rule consensus tree was computed with posterior probability (PP) and branch length averaged for all nodes. The ML and BI consensus trees were visualized using FigTree 1.4.2 (Rambaut, 2014). All data sets and phylogenetic trees are deposited in TreeBASE (Study ID: S24023). The distribution map was prepared using ArcGIS Pro version 2.5.0 (ESRI, 2020).

We also provide a key to the 11 species of *Lellingeria* thus far known to occur in Panama and neighboring Costa Rica. *Lellingeria vargasiana* A. Rojas, from Cocos Islands, is considered here as a synonym of *L. tmesipteris* (Copel.) A.R.Sm. & R.C.Moran.

Results

Phylogenetic Analysis.—Analysis of the concatenated cpDNA produced a monophyletic *Lellingeria* clade in both the ML and BI analyses, each with the same topology and strong branch support (PP = 1; BS = 100). Consensus tree topology (Fig. 3) recovered the same major clades as previous studies (Bauret *et al.*, 2017; Labiak, Sundue, and Rouhan, 2010; Ranker *et al.*, 2004; Shalisko *et al.*, 2019). Our analyses of each individual marker, as well as the concatenated dataset, support the new species as a member of *Lellingeria,* as currently recognized. Our results resolved the new species on a long branch with strong support and sister to the *L. suprasculpta* clade (PP = 1; BS = 100). We find no evidence for a close relationship to either *L. kaieteura*, *L. subimpressa*, or *L. epiphytica*, as morphology would suggest.

Discussion

Rapid and repetitive character evolution has been documented in grammitid ferns resulting in superficial similarities between species and genera (Ranker *et al*, 2004; Sundue, 2010). However, *L. cantarensis* displays the salient character states of the genus: clathrate and ciliate rhizome scales, veins ending in hydathodes, and indument comprising minute asymmetrically branched hairs bearing hyaline acicular marginal cilia (Labiak, 2013); it is not likely to be confused at the genus level. Like other *Lellingeria* species, it lacks the reddish pluricellular setae found in most grammitids (Sundue, 2010). It is distinct from its congeners by its short lamina (6 cm or less) with a truncate base, oblique pinnae, and the abaxial side of rachis is densely pubescent. *Lellingeria*

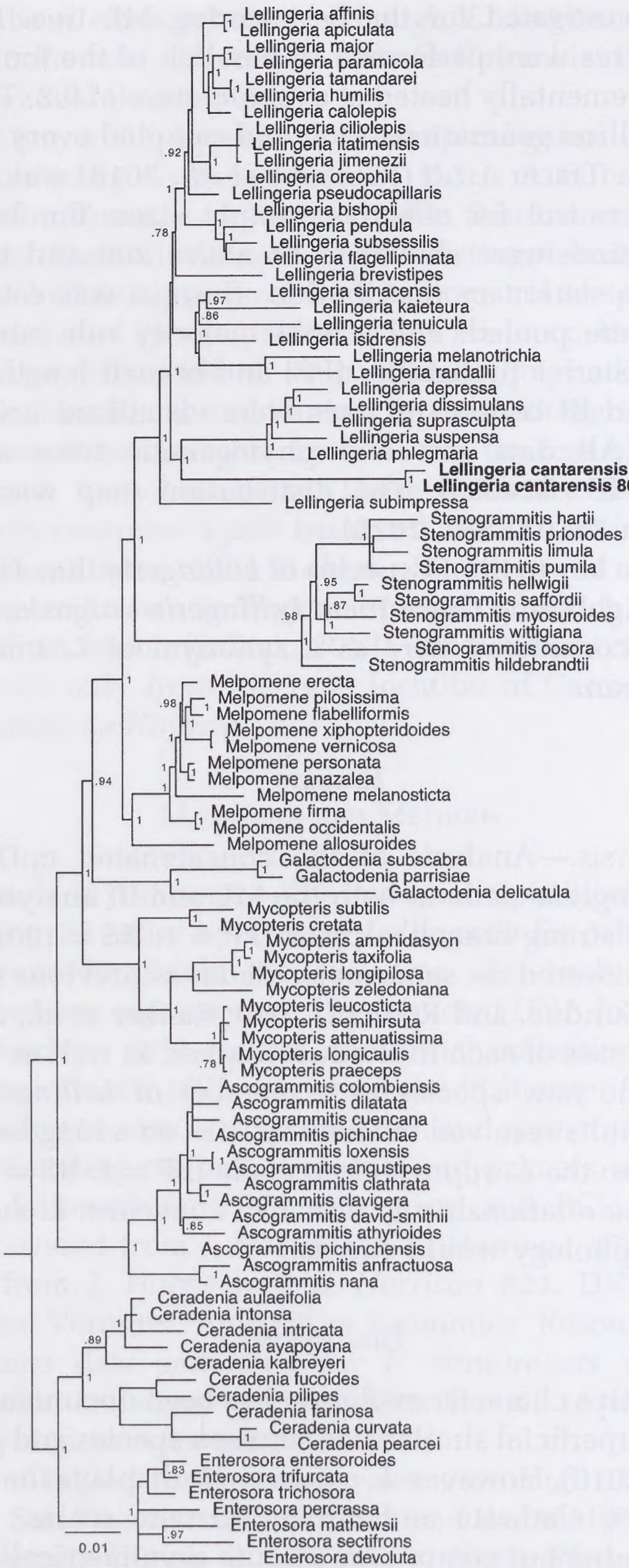

FIG. 3. Majority rule consensus phylogram from Bayesian analysis using the concatenated dataset from all five plastid DNA markers (*atpB*, *rbcL*, *trnG-trnR*, *trnL-trnF*, and *rps4-trnS)*. Posterior probability (PP) is represented by numerical values at each branch. PP values equal to 1 are indicated with an asterisk. The number of nucleotide substitutions per site is indicated by the scale bar.

cantarensis is likely to be mistaken for *L. kaieteura* or *L. epiphytica,* both of which also have clathrate rhizome scales with ciliate margins, a pubescent abaxial surface of the rachis, and slightly oblique segments. However, *L. cantarensis* can be distinguished from *L. kaieteura* by having conspicuously oblique segments ascending 60°–70° to the rachis, a thicker blade texture with less deeply sunken sori, shorter and narrower rhizome scales, and a truncate laminar base. *Lellingeria epiphytica* differs from *L. cantarensis* by possessing a flexuous rachis with deeply sunken sori. *Lellingeria cantarensis* could also be confused for *L. subimpressa* and *L. apiculata*, which share the truncate laminar base. However, *L. subimpressa* differs by an abaxially glabrous rachis, and *L. apiculata* by its larger fronds and rhizome scales. These characters, the small laminae of *L. cantarensis,* and its restricted locality should distinguish it from all other species in the genus as well.

The dwarf size of *L. cantarensis* is distinctive and may have led to it being overlooked. When compared to most other ferns, grammitids tend to be small plants (Parris, 1990). Yet, with laminae less than 6 cm long (only 2.2–3.2 cm long in *J. Harrison & L. Harrison 865*), *L. cantarensis* is minute, even among grammitids. Repeated patterns of morphological dwarfism have been demonstrated to have evolved within the grammitids (Testo and Sundue, 2018). Schneider *et al.* (2004) surmised that dwarfism was a driver of rapid radiation in grammitid ferns, but support for this has not been found (Sundue, Testo, and Ranker, 2015). Instead, it appears that the repeated evolution of small plants is related to niche occupation within epiphytic habitats (Aros-Mualin *et al.,* 2020; Nitta, Watkins, and Davis, 2020).

TAXONOMIC TREATMENT

Lellingeria cantarensis B.K. Black & Sundue, **sp. nov.**

TYPE: PANAMA. Panama: Los Altos de Cerro Azul, Cerro Jefe Region, 9° 13′ 50” N, 79° 24′ 13” W, El Cantar Trail near second major footbridge, 0.75 km from El Fortín trailhead at the end of Calle Andorra, 818 m, 07 May 2019, *J. Harrison and L. Harrison 865* (holotype: VT!) (Fig. 4, A–C).

Plants epiphytic; **fronds** spreading to pendent, determinate; **rhizomes** decumbent to erect, radially symmetrical, with ventral root insertion, scaly; **scales** dark brown, 0.8–1.0 mm × 0.3–0.5 mm, lanceolate, apices short-attenuate, the margins ciliate; **petioles** 1.2–1.4 cm × 0.5 mm, dark brown, terete, densely pubescent, the hairs 0.1–0.2 mm long, spreading, simple, 1-celled, acicular, hyaline; **laminae** 2.2–5.1 cm long, oblong, chartaceous, pinnate, with 9–12 pairs of segments, the bases truncate, proximal pinnae not basiscopically decurrent onto petiole base, slightly broader in the middle, apex gradually reduced, pinnatifid, ending in a short conform or subconform segment; **rachises** straight, with dark sclerenchyma visible throughout their length, provided on both sides with hyaline hairs, adaxially the hairs 0.1–0.3 mm long, simple and 1-celled, and abaxially with hairs 0.1–0.3 mm long, 1–2-furcate and multi-celled; **segments** ca. 3–5 × 0.5–1 mm, plane, ascending 60° to

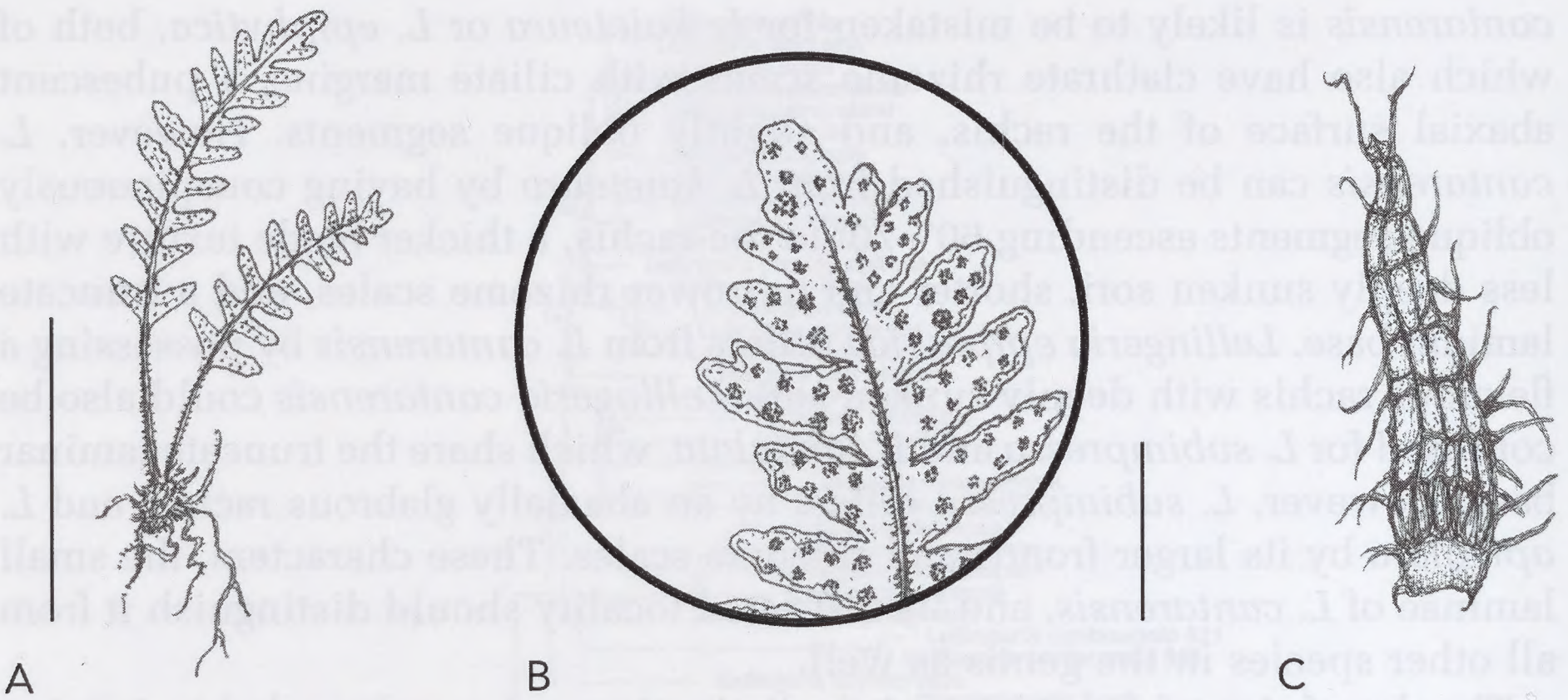

FIG. 4. *Lellingeria cantarensis*. A. habit, *J. Harrison and L. Harrison 865* (VT); scale bar = 3 cm. B. Adaxial surface of distal part of lamina, *J. Harrison and L. Harrison 865* (VT); scale bar = 0.5 cm. C. Detail of rhizome scale with ciliate margin, *J. Harrison and L. Harrison 865* (VT).

the rachis, widened and asymmetric at the base, distal pinnae decurrent basiscopically, cuneate acroscopically, the base attached parallel on the rachis (*i.e.*, both sides inserted along the same radius), not overlapping the base of the next segment, apices obtuse, margins entire, slightly recurved, abaxial laminar surfaces sparsely pubescent, hairs similar to those of the rachises, adaxial laminar surfaces glabrous; **sinuses** narrower than segments; **venation** pinnate with 6–10 pairs of veins per segment; **costae** with black sclerenchyma obscured by laminar tissue on both sides, sparsely pubescent with hairs similar to those of the rachis; **veins** simple, with blackish sclerenchyma, ending in hydathodes that lack calcareous deposits; **sori** medial, rounded, sometimes overlapping costae but not pinna margins, 4(–5) pairs per segment, superficial to slightly sunken; **sporangia** capsules glabrous.

Additional Specimen Examined. PANAMA. Panama: Cerro Jefe Region. 9.23055556°, -79.40361111°, Los Altos de Cerro Azul, El Cantar Trail near second major footbridge, ca. 0.75 km from El Fortín trailhead at end of Calle Andorra, 818 m, 15 Jan 2019, *J. Harrison & L. Harrison 821* (UC).

Diagnosis.—The new species differs from all other species of *Lellingeria* by its minute laminae less than 6 cm long, truncate laminar base, and the abaxial side of rachises are densely pubescent.

Distribution and Ecology.—Epiphytes, growing on low mossy trunks in the premontane forests of Chagres National Park in Panama. Plants have been found growing near creeks in Los Altos de Cerro Azul, alongside *Tectaria athyrioides* C.Chr. and *Selaginella mollis* A.Br. Although extensive collections of this region have been made in the past century, this species has not been found at nearby sites, such as Cerro Azul or the surrounding Altos de Pacora foothills; only two collections are known, both from the same location.

Etymology.—The new species is named for the Cantar Trail, located in the foothills of Cerro Jefe, in Panama Province, Panama.

Key to the Lellingeria of Panama and Costa Rica

1. Rhizome scales glabrous or each with a single apical cilium
   2. Rachises greenish (dark sclerenchyma covered by the laminar tissue) . . . . *L. phlegmaria*
   2. Rachises blackish (the dark sclerenchyma not covered by the laminar tissue)
      3. Segments linear-deltate, deeply pinnatifid, acute to mucronate . . . . *L. suprasculpta*
      3. Segments linear to linear-lanceolate, entire or slightly crenulate (never pinnatifid nor mucronate) . . . . . *L. suspensa*
1. Rhizome scales ciliate throughout
   4. Segments deeply incised to crenate, the apices acute to mucronate
      5. Fronds usually more than 15 cm long; sori sunken . . . . . *L. micula*
      5. Fronds up to 10(–12) cm long; sori superficial . . . . . *L. randallii*
   4. Segments entire or crenulate, the apices rounded or obtuse
      6. Laminae truncate at their bases; basal segments about half the length of the medial ones (not reduced to auricles)
         7. Rachises glabrescent abaxially; costae visible on the abaxial side (dark sclerenchyma visible) . . . . . *L. subimpressa*
         7. Rachises pubescent abaxially; costae often obscured by the laminar tissue abaxially (dark sclerenchyma not visible)
            8. Fronds to 6 cm long; rhizome scales 0.8–1 mm long . . . . . *L. cantarensis*
            8. Fronds longer than 12 cm (usually 15–20 cm long); rhizome scales 2–3 mm long . . . . . *L. apiculata*
      6. Laminae gradually reduced to their bases, the lowermost segments reduced to auricles
         9. Rachises greenish (their dark sclerenchyma covered by a green, laminar tissue) . . . . . *L. subsessilis*
         9. Rachises blackish (their dark sclerenchyma exposed on both sides)
            10. Rhizome scales less than 0.5 mm long; sori deeply sunken . . . . *L. isidrensis*
            10. Rhizome scales longer than 1 mm; sori superficial or only slightly sunken
               11. Segments deltate to linear-deltate, expanded at the base . . . . *L. barbensis*
               11. Segments linear to lanceolate, slightly constricted at the base . . . . . *L. tmesipteris*

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Literature Cited

Almeda, F., A. Marcela, and H. Mendoza. 2014. Two new species of *Graffenrieda* (Melastomataceae: Merianieae) from Colombia and Panama. Phytotaxa 163:39–47.

ANAM. 2000. Mapa de vegetación de Panamá. ANAM, Corredor Biológico Mesoamericano del Atlántico Panameño, Louis Berger Group, Intl., basado en el sistema de clasificación de la UNESCO (Mueller-Dombois & Ellenberg, 1974).

Aros-Mualin, D., S. Noben, D. N. Karger, C. I. Carvajal-Hernández, L. Salazar, A. Hernández-Rojas, J. Kluge, M. A. Sundue, M. Lehnert, D. Quandt, and M. Kessler. 2020. Functional diversity in ferns is driven by species richness rather than by environmental constraints. Frontiers in Plant Science 11:21–28.

Bauret, L., M. Gaudeul, M. A. Sundue, B. S. Parris, T. A. Ranker, F. Rakotondrainibe, S. Hennequin, J. Ranaivo, M. A. Selosse, and G. Rouhan. 2017. Madagascar sheds new light on the molecular

systematics and biogeography of grammitid ferns: New unexpected lineages and numerous long-distance dispersal events. Molecular Phylogenetics and Evolution 111:1–17.

Berry, P. E., and C. Galdames. 2013. *Croton cerroazulensis* (Euphorbiaceae), a new species from Cerro Azul (Panama). Webbia 68:17–19.

De Sedas, A., L. Martinez, M. Stapf, and M. Correa Arroyo. 2009. *Guía Fotográfica de las Plantas Vasculares de Cerro Jefe, Panamá.* Instituto Nacional de Biodiversidad, INBio, Santo Domingo.

Dick, C. W., R. Condit, and E. Bermingham. 2005. Biogeographic history and the high beta-diversity of rainforest trees in Panamá. Pp. 259–269, *in* R. S. Harmon (ed.), *The Río Chagres, Panama: AMultidisciplinary Perspective of a Tropical River Basin.* Springer, Dordrecht.

ESRI. 2020. ArcGIS Pro (Version 2.5). Esri Inc. https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview.

Empresa de Transmisión Eléctrica S.A. (ETESA). 2008. Gerencia de Hidrometeorología, Panamá.

Harmon, R. S. 2005. The geological development of Panama. Pp. 45–62, *in* R. S. Harmon (ed.), *The Río Chagres: A Multidisciplinary Perspective of a Tropical River Basin.* Springer, Dordrecht.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30:772–780.

Kesler, S. E., J. F. Sutter, M. J. Issigonis, L. M. Jones, and R. L. Walker. 1977. Evolution of porphyry copper mineralization in an oceanic island arc; Panama. Economic Geology 72:1142–1153.

Kuijt, J., J. Harrison, and L. Harrison. 2015. Endemism in two new species of *Dendrophthora* (Viscaceae) from Cerro Jefe, Panama. Phytologia 97:139–144.

Labiak, P. H., M. Sundue, and G. Rouhan. 2010. Molecular phylogeny, character evolution, and biogeography of the grammitid fern genus *Lellingeria* (Polypodiaceae). American Journal of Botany 97:1354–1364.

Labiak, P. H. 2013. Lellingeria. Pp.1-130, *in* T. Zanoni (ed), *Flora Neotropica. Volume 111. Grammitid ferns (Polypodiaceae*). New York Botanical Garden, New York.

Maury, R. C., M. J. Defant, H. Bellon, R. H. Stewart, and J. Cotte. 1995. Early Tertiary arc volcanics from eastern Panama. Pp. 29–34, *in* P. Mann (ed.), *Geologic and Tectonic Development of the Caribbean Plate Boundary in Southern Central America.* Geologic Society of America, Special Paper 295.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2011. The CIPRES science gateway: a community resource for phylogenetic analyses. Proceedings of the 2011 TeraGrid Conference: extreme digital discovery, 1–8.

Nitta, J. H., J. E. Watkins Jr, and C. C. Davis. 2020. Life in the canopy: community trait assessments reveal substantial functional diversity among fern epiphytes. New Phytologist 227:1885–1899.

Parris, B. S. 1990. Grammitidaceae. Pp. 153–157, *in* K. U. Kramer, and P. S. Green (eds.), *The Families and Genera of Vascular Plants. Volume 1. Pteridophytes and Gymnosperms.* Springer-Verlag, Berlin.

PPG I. 2016. A community-derived classification for extant lycophytes and ferns. Journal of Systematics and Evolution 54:563–603.

Rambaut, A. 2014. FigTree 1.4. 2 software. Institute of Evolutionary Biology, University of Edinburgh.

Rambaut, A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901–904.

Ranker, T. A., A. R. Smith, B. S. Parris, J. M. Geiger, C. H. Haufler, S. C. Straub, and H. Schneider. 2004. Phylogeny and evolution of grammitid ferns (Grammitidaceae): a case of rampant morphological homoplasy. Taxon 53:41–428.

Ronquist, F., M. Teslenko, P. van Der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

Schneider, H., A. R. Smith, R. Cranfill, T. J. Hildebrand, C. H. Haufler, and T. A. Ranker. 2004. Unraveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): exploring aspects of the diversification of epiphytic plants. Molecular Phylogenetics and Evolution 31:1041–1063.

Shalisko, V., M. A. Sundue, A. R. Villalobos-Arambula, M. Á. Muniz-castro, and J. A. Vazquez-Garcia. 2019. Taxonomic novelties in grammitid ferns (Polypodiaceae) from the Neotropics and Madagascar supported by molecular data. Phytotaxa 394:176–208.

Smith, A. R., R. C. Moran, and L. E. Bishop. 1991. *Lellingeria*, a new genus of Grammitidaceae. American Fern Journal 81:76–88.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Sundue, M. A. 2010. A morphological cladistic analysis of *Terpsichore* (Polypodiaceae). Systematic Botany 35:716–729.

Sundue, M. A. 2019. Three new species of grammitid ferns (Polypodiaceae) from the fern hunter's paradise: Sierra Juárez, Oaxaca, Mexico. Systematic Botany 42:160–168.

Sundue, M. A., M. B. Islam, and T. A. Ranker. 2010. Systematics of grammitid ferns (Polypodiaceae): using morphology and plastid sequence data to resolve the circumscriptions of *Melpomene* and the polyphyletic genera *Lellingeria* and *Terpsichore*. Systematic Botany 35:701–715.

Sundue, M. A., B. S. Parris, T. A. Ranker, A. R. Smith, E. L. Fujimoto, D. Zamora-Crosby, C. W. Morden, W. L. Chiou, C. W. Chen, G. Rouhan, and R. Y. Hirai. 2014. Global phylogeny and biogeography of grammitid ferns (Polypodiaceae). Molecular Phylogenetics and Evolution 81:195–206.

Sundue, M. A., W. L. Testo, and T. A. Ranker. 2015. Morphological innovation, ecological opportunity, and the radiation of a major vascular epiphyte lineage. Evolution 69:2482–2495.

Testo, W. L. and M. A. Sundue. 2018. Are rates of species diversification and body size evolution coupled in the ferns? American Journal of Botany 105:525–535.

Appendix 1. GenBank accession numbers for DNA sequences used in this study. Sequences generated as part of this study are in boldface.

Species	Voucher	Accession Number				
		atpB	*rbcL*	*rps4-trnS*	*trnG-trnR*	*trnL-trnF*
Ascogrammitis anfractuosa (Kunze ex Klotzsch) Sundue	Jimenz 1399	GU476779	GU476833	–	KM105967	GU476672
Ascogrammitis angustipes (Copel.) Sundue	Rodriguez 5124	–	GU476891	–	KM105968	GU476702
Ascogrammitis athyrioides (Hook.) Sundue	Lehnert 261	–	GU476856	–	KM105969	GU476704
Ascogrammitis clathrate (Sundue & M.Kessler) Sundue	Kromer 1237	–	GU476843	–	KM105970	GU476708
Ascogrammitis clavigera A.R.Sm. ex Sundue	Schneider 2400	–	GU476925	–	–	GU476709
Ascogrammitis colombiensis Sundue	Sundue & Vasco 1316	GU476804	GU476827	–	–	GU476710
Ascogrammitis cuencana (Hieron.) Sundue	Lehnert 1164	–	GU476851	–	KM105971	GU476714
Ascogrammitis david-smithii (Stolze) Sundue	Sundue 785	GU376639	GU387012	–	GU387205	GU387284
Ascogrammitis dilatata (Sundue & M.Kessler) Sundue	Labiak 4728	GU376640	GU387033	–	GU387206	GU387285
Ascogrammitis loxensis Sundue	Sundue 1073	GU376641	GU386995	–	GU387207	GU387286
Ascogrammitis nana (Sundue & M.Kessler) Sundue	Labiak 4725	GU376642	GU387031	–	GU387208	GU387287
Ascogrammitis pichinchae (Sodiro) Sundue	K. A. Wilson 2816a	–	GU476928	–	–	GU476730
Ascogrammitis pichinchensis (Hieron.) Sundue	Rodriguez 646	GU476816	GU476854	–	–	GU476731
Ceradenia aulaeifolia L.E.Bishop ex A.R.Sm.	A. Rojas 3232	GU476748	GU476902	–	KM105974	GU476623
Ceradenia ayapoyana M.Kessler & A.R.Sm.	A. Rojas & al. 3232	–	–	–	KM105975	–
Ceradenia curvata (Sw.) L.E.Bishop	I. Jimenez 1559	–	–	–	KM105976	–
Ceradenia farinosa (Hook.) L.E.Bishop	David Neill 11985	–	–	–	KM105977	–
Ceradenia fucoides (Christ) L.E.Bishop	Sundue 1766	GU476749	GU476907	–	–	GU476625
Ceradenia intonsa L.E.Bishop ex León-Parra & Mostacero	Sundue & Vasco 1321	GU476750	GU476901	–	–	–
Ceradenia intricata (C.V.Morton) L.E.Bishop *ex* A.R.Sm.	Lehnert 1108	–	–	–	KM105978	GU476626
Ceradenia kalbreyeri (Baker) L.E.Bishop	Sundue 1759	GU476744	GU476887	–	KM105979	GU476617
Ceradenia pearcei (Baker) L.E.Bishop	A.F. Fuentez & Villalobos 13997	–	–	–	KM105980	–
Ceradenia pilipes (Hook.) L.E.Bishop	A. Rojas 3233	GU476746	GU476869	–	–	GU476620
Enterosora enterosoroides (Christ) A.Rojas	Sundue 1172	GU476756	–	–	–	GU476634

Appendix Continued.

Species	Voucher	Accession Number				
		atpB	*rbcL*	*rps4-trnS*	*trnG-trnR*	*trnL-trnF*
Enterosora percrassa (Baker) L.E.Bishop	Sundue 1165	GU476757	GU476882	–	–	GU476635
Enterosora trichosora (Hook.) L.E.Bishop	Moran 7569	–	GU476920	–	–	GU476637
Enterosora trifurcata (L.) L.E.Bishop	Sundue 1774	GU476754	GU476909	–	–	GU476632
Galactodenia parrisiae Sundue & Labiak	Monro & Knapp 5721	–	–	–	KM105990	–
Galactodenia subscabra (Klotzsch) Sundue & Labiak	Moran 8078	–	GU476860	GU387127	GU387209	GU476739
Lellingeria affinis Labiak	Jimenez 368	GU376570	GU386982	GU387044	GU387130	GU387212
Lellingeria apiculata (Klotzsch) A.R.Sm. & R.C.Moran	Labiak 3884	GU376572	GU387021	GU387046	GU387132	GU387214
Lellingeria bishopii Labiak	Sundue 1159	GU376574	GU387000	GU387048	GU387134	GU387216
Lellingeria brevistipes (Kuhn) A.R.Sm. & R.C.Moran	Labiak 3633	GU376575	GU387018	GU387049	GU387135	GU387217
Lellingeria calolepis Labiak	Jimenez 1731	GU376577	GU386979	GU387051	GU387137	GU387219
Lellingeria ciliolepis (C.Chr.) A.R.Sm.	Labiak 2904	GU376578	GU387016	GU387052	GU387138	GU387220
Lellingeria depressa (C.Chr.) A.R.Sm. & R.C.Moran	Labiak 3570	GU376579	GU387017	GU387053	GU387139	GU387221
Lellingeria dissimulans (Maxon) A.R.Sm.	Williams 40423	GU376580	GU387043	GU387054	GU387140	GU387222
Lellingeria flagellipinnata M.Kessler & A.R.Sm.	Labiak 2866	GU376581	GU387015	GU387055	GU387141	GU387223
Lellingeria humilis (Mett.) A.R.Sm. & R.C.Moran	Sundue & Vasco 1289	–	GU476897	GU387060	GU387146	GU476649
Lellingeria isidrensis (Maxon ex Copel.) A.R.Sm. & R.C.Moran	Wagner 83020	GU376586	GU387042	GU387061	GU387147	GU387228
Lellingeria itatimensis (Copel.) A.R.Sm. & R.C.Moran	Labiak 3872	GU376587	GU387020	GU387062	GU387148	GU387229
Lellingeria jimenezii Labiak	Lehnert 466	GU376588	GU386981	GU387063	GU387149	GU387230
Lellingeria kaieteura (Jenman) Labiak	Hombersley 120	GU376590	GU386978	GU387065	GU387151	GU387231
Lellingeria major (Copel.) A.R.Sm. & R.C.Moran	Sundue 1147	GU376592	GU386998	GU387068	GU387154	GU387233
Lellingeria melanotrichia (Baker) A.R.Sm. & R.C.Moran	Contreras 300	GU376593	GU386973	GU387070	GU387156	GU387234
Lellingeria oreophila (Maxon) A.R.Sm. & R.C.Moran	Contreras 159	–	GU386974	GU387080	GU387165	GU387243
Lellingeria paramicola Labiak	Øllgaard 8403	GU376601	GU387014	GU387081	GU387166	GU387244
Lellingeria pendula (Sw.) A.R.Sm. & R.C.Moran	Meier 706	GU376602	GU387036	GU387082	GU387167	GU387245

APPENDIX Continued.

Species	Voucher	Accession Number				
		atpB	*rbcL*	*rps4-trnS*	*trnG-trnR*	*trnL-trnF*
Lellingeria phlegmaria (J.Sm.) A.R.Sm. & R.C.Moran	Sundue 1345	GU376603	GU387008	GU387084	GU387168	GU387247
Lellingeria pseudocapillaris (Rosenst.) A.R.Sm. & R.C.Moran	Jimenez 1800	GU376606	GU386980	GU387087	GU387171	GU387250
Lellingeria randallii (Maxon) A.R.Sm. & R.C.Moran	Mickel 9495	GU376609	GU386992	GU387090	GU387174	GU387253
Lellingeria simacensis (Rosenst.) A.R.Sm. & R.C.Moran	Sundue 1169	GU376610	GU387001	GU387092	GU387175	GU387255
Lellingeria cantarensis Black & Sundue	J. & L. Harrison 865	**MZ147036**	**MZ147035**	**MZ147037**	**MZ147038**	**MZ147039**
Lellingeria cantarensis Black & Sundue	J. & L. Harrison 821	**MZ127822**	–	**MZ147034**	–	–
Lellingeria subimpressa (Copel.) Labiak	Arauz 368	GU376611	GU386967	GU387093	GU387176	GU387256
Lellingeria subsessilis (Baker) A.R.Sm. & R.C.Moran	Sundue 1124	GU376612	GU386996	GU387094	GU387177	GU387257
Lellingeria suprasculpta (Christ) A.R.Sm. & R.C.Moran	Lellinger 989	GU376614	GU386987	GU387096	GU387179	GU387259
Lellingeria suspensa (L.) A.R.Sm. & R.C.Moran	Vasco 632	GU376615	GU386969	GU387097	GU387180	GU387260
Lellingeria tamandarei (Rosenst.) A.R.Sm. & R.C.Moran	Labiak 4430	GU376621	GU387027	GU387103	GU387186	GU387266
Lellingeria tenuicula (Fée) A.R.Sm. & R.C.Moran	Hill 28165	GU376622	GU386977	GU387104	GU387187	GU387267
Melpomene allosuroides (Rosenst.) A.R.Sm. & R.C.Moran	Solomon 12829	GU376628	GU387038	GU387109	GU387192	GU387273
Melpomene anazalea Sundue & Lehnert	Sundue & Vasco 1290	GU476773	GU476898	–	KM105997	GU476662
Melpomene erecta (C.V.Morton) A.R.Sm. & R.C.Moran	Nunez 25	GU376629	GU387013	GU387110	GU387193	GU387274
Melpomene firma (J.Sm.) A.R.Sm. & R.C.Moran	Sundue 1253	GU376630	GU387003	GU387111	GU387194	GU387275
Melpomene flabelliformis (Poir.) A.R.Sm. & R.C.Moran	Sundue 1303	GU376631	GU387005	GU387113	GU387196	GU387277
Melpomene melanosticta (Kunze) A.R.Sm. & R.C.Moran	Labiak 4156	GU376633	GU387024	GU387115	GU387198	GU387279
Melpomene occidentalis Lehnert	Sundue 1168	GU476775	GU476877	–	–	GU476665
Melpomene personata Lehnert	Sundue 1330	GU376634	GU387007	GU387116	GU387199	GU387280
Melpomene pilosissima (M.Martens & Galeotti) A.R.Sm. & R.C.Moran	Martinez 1535	GU376636	GU386993	GU387118	GU387201	GU387281

Appendix Continued.

Species	Voucher	Accession Number				
		atpB	*rbcL*	*rps4-trnS*	*trnG-trnR*	*trnL-trnF*
Melpomene vernicosa (Copel.) A.R.Sm. & R.C.Moran	Sundue 1112	GU476778	GU476867	–	–	GU476668
Melpomene xiphopteroides (Liebm.) A.R.Sm. & R.C.Moran	Sundue 1300	GU376637	GU387004	GU387119	GU387202	GU387282
Mycopteris amphidasyon (Kunze ex Mett.) Sundue	Sundue 1129	GU476759	GU476872	–	KM106007	GU476638
Mycopteris attenuatissima (Copel.) Sundue	Sundue 1098	–	GU476850	GU387121	GU387204	GU476705
Mycopteris cretata (Maxon) Sundue	Alford 2719	–	GU476823	–	–	GU476713
Mycopteris leucosticta (J.Sm.) Sundue	Lehnert 1128	GU476810	GU476848	–	–	GU476719
Mycopteris longicaulis (Sundue & M.Kessler) Sundue	Jimenez 373	GU476813	GU476840	–	–	GU476724
Mycopteris longipilosa Sundue	Sundue & Martin 1033	GU476814	GU476861	–	KM106008	GU476725
Mycopteris praeceps (Sundue & M.Kessler) Sundue	Jimenez 2173	GU476817	GU476839	–	–	GU476734
Mycopteris semihirsuta (Klotzsch) Sundue	Sundue & Vasco 1246	GU476818	GU476894	–	–	GU476735
Mycopteris subtilis (Kunze ex Klotzsch) Sundue	Lehnert 1396	GU376643	GU386984	GU387128	GU387210	GU387288
Mycopteris taxifolia (L.) Sundue	Sundue & Werner 1161	GU476792	GU476880	–	KM106009	GU476686
Mycopteris zeledoniana (Lellinger) Sundue	Sundue 1806	–	GU387011	GU387129	GU387211	–
Stenogrammitis hartii (Jenman) Labiak	Lellinger 455	GU376583	GU386985	GU387057	GU387143	GU387225
Stenogrammitis hellwigii (Mickel & Beitel) Labiak	Mickel & Leonard 4636	GU376584	GU386990	GU387058	GU387144	GU387226
Stenogrammitis hildebrandtii (Hieron.) Labiak	Rakotondrainibe 6112	GU376585	GU386975	GU387059	GU387145	GU387227
Stenogrammitis limula (Christ) Labiak	Sundue 1736	GU376591	GU387023	GU387066	GU387152	GU387232
Stenogrammitis myosuroides (Sw.) Labiak	Vasco 631	GU376594	GU386968	GU387071	GU387157	GU387235
Stenogrammitis oosora (Baker) Labiak	Cable 77	GU376600	GU386972	–	GU387164	GU387241
Stenogrammitis prionodes (Mickel & Beitel) Labiak	Mickel 6567	GU376605	GU386991	GU387086	GU387170	GU387249
Stenogrammitis pumila (Labiak) Labiak	Labiak 4015	GU376608	GU387022	GU387089	GU387173	GU387252
Stenogrammitis saffordii (Maxon) Labiak	Ranker 1892	–	GU476926	–	–	GU476656
Stenogrammitis wittigiana (Fée & Glaz.) Labiak	Sylvestre 1885	GU376623	GU386989	GU387073	GU387159	GU387268
Zygophlebia devoluta (Baker) Parris	Kluge 7940	–	–	–	KM106041	–
Zygophlebia mathewsii (Mett.) L.E.Bishop	Sundue & Vasco 1254	–	GU476868	–	KM106043	GU476743
Zygophlebia sectifrons (Kunze ex Mett.) L.E.Bishop	Sundue 1757	–	GU476904	–	KM106044	–

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Morphological Plasticity in the Endemic *Isoëtes* Species from Serra dos Carajás, Amazonia, Brazil

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Abstract.—*Isoëtes cangae* and *Isoëtes serracarajensis* (Isoetaceae) are species endemic to Carajás (Pará State, Brazil) that grow on iron-rich substrates of the Amazon basin under a seasonal rainfall regime. In addition to a few distinctive morphological traits, the species differ in terms of habitat and geographical distribution. The influence of the seasonality and habitat characteristics on quillwort morphology is poorly understood. To assess this influence, specimens from one population of *I. cangae*, and four populations of *I. serracarajensis*, were sampled in dry and rainy seasons to investigate variation among vegetative and reproductive characters. The megasporangium color, megaspore ornamentation, alae length, velum length and coverage (%), morphometry of sporangial wall cells, and polar/equatorial diameter ratio of megaspores provide consistent characters for species delimitation. The presence of stomata in *I. serracarajensis* is a new discovery and is related to its amphibious habit. Some characters that have been used in *Isoëtes* species descriptions, such as the number of leaves, leaf length, sporangium size, number of megaspores, and megaspore diameter were found to be variable. The current findings provide further information on the biology of the species and show the influence of seasonality and habitat characteristics on the morphological diversity of these lycophytes.

Key Words.—lycophyte, morphometry, megaspores, stomata, seasonality

Isoëtes L. is a lycophyte genus in the family Isoetaceae, which comprises about 250 cosmopolitan species (Troia *et al.*, 2016). Most species are aquatic, living in lakes, rivers, ponds, streams, estuaries, and swamps, although several are terrestrial, living in seasonally flooded areas (Page, 2002; Sharpe, Mehltreter, and Walker, 2010; Taylor and Hickey, 1992). About 29 species in the genus have been described in Brazil (Pereira, Stützel, and Schulz, 2017;

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Pereira, Guimarães, and Watanabe, 2019; Troia *et al.*, 2016), but many of these species are known from a limited number of collections, and distinguishing among the taxa is challenging (Pereira and Labiak, 2013).

Isoëtes cangae J.B.S. Pereira, Salino & Stützel and *Isoëtes serracarajensis* J.B.S. Pereira, Salino & Stützel are endemic to the Serra dos Carajás, southeastern Pará, Brazil. Serra dos Carajás is a mountain range with altitudes above sea level of 600-760 m and it holds the world's largest iron ore reserve (Giulietti *et al.*, 2019). Four main ferruginous formations make up the Serra dos Carajás: North, South, and East ranges (Serra Norte, Serra Sul, and Serra Leste), and Serra de São Félix (Viana *et al.*, 2016). Several ponds formed by the structural and degradation process of the lateritic profile are found on the higher parts of the plateaus (Maurity and Kotschoubey, 1995). The rupicolous vegetation that develops over the lateritic crusts, called *canga*, is found in association with the water bodies (Guimarães *et al.*, 2019).

Isoëtes cangae is among the eight species found exclusively in Carajás National Forest (Giulietti *et al.*, 2019). It is a submerged aquatic that occurs in a single permanent pond in the South mountain range. In contrast, *I. serracarajensis* is an amphibious aquatic found in several seasonal flooded areas in the North, South, Tarzan, Cristalino, and Bocaina mountain ranges (Nunes *et al.*, 2018; Pereira, Arruda, and Salino, 2017).

Both species have been described recently and can be distinguished from other species of the genus by morphological characters such as leaf shape, velum coverage, sporangium wall color, and spore size and ornamentation (Pereira *et al.*, 2016; Pereira, Arruda, and Salino, 2017). Genetic analysis using DNA barcoding and the chloroplast genome indicate that *I. cangae* and *I. serracarajensis* are very similar, but can be distinguished by quantitative assessment of morphological elements of the megaspore (Nunes *et al.*, 2018). However, the morphological differences between species require more detailed investigations, given the phenotypic plasticity and seasonal variation common to species in the group. For instance, *I. serracarajensis* specimens collected in flooded areas along the ferruginous mountain ranges vary in leaf size and morphology, indicating the importance of studying individual populations. In addition, Inter Simple Sequence Repeat (ISSR) molecular markers group the *I. serracarajensis* populations geographically and indicate significant differences between the populations from the North Range and the South Range (Santos *et al.*, 2020).

Phenotypic plasticity is the ability of a given genotype to express distinct phenotypes in response to different environments (Sultan, 2000). Most examples of phenotypic plasticity are known from aquatic and amphibious macrophytes compared to terrestrial forms (Barrett, Eckert, and Husband, 1993; Wells and Pigliucci, 2000). Factors such as water body depth, light, nutrient availability, and population density may affect the plant's growth and life cycle. Various aquatic species occur in habitats with strong environmental gradients, and frequently display considerable morphological modification in response to varying water levels (Dorken and Barrett, 2004). Without such plasticity, the species might not adapt to these rapid variations, and tolerance

to deeper waters might depend on the plant's height or age (Clevering *et al.*, 1998).

Phenotypic plasticity is evident among *Isoëtes* species (Vöge, 2003). Liu, Wang, and Taylor (2006) investigated three *I. sinensis* Palmer populations and found considerable variation in leaf length, leaf number, and spore size associated with differences in climate and location. Another study of six populations of *I. panchganiensis* G.K.Srivast., D.D.Pant & P.K.Shukla obtained similar results (Yadav *et al.*, 2012). Size and ornamentation of megaspores can slightly vary among populations of the same species, which may corroborate the definition of subspecies or varieties (Hall, 1971; Marsden, 1976; Takamiya, Watanabe, and Ono, 1997). Knowing the range of the morphological variation in the different populations helps in distinguishing among species (Yadav *et al.*, 2012).

Given that phenotypic plasticity occurs among *I. cangae* and *I. serracarajensis* populations, which may owe to both the ecological variation of their habitats and documented genetic differences among and within the species (Santos *et al.*, 2020), the objective of the present study was to investigate the seasonal and geographical variation in the morphology and morphometry of vegetative and reproductive organs of *Isoëtes* species endemic to the Carajás canga. Knowledge concerning phenotypic plasticity of these sympatric species is essential for taxonomic determination, for understanding of their biology, and for conservation of macrophyte diversity.

Material and Methods

Plant collection.—Specimens of *Isoëtes cangae* and *Isoëtes serracarajensis* were collected in Serra dos Carajás (PA), southeastern Pará State, within the boundaries of the Carajás National Forest (05°52′00″ - 06°33′00″S, 049° 53′00″ - 050°45′00″W) (Fig 1). The climate of the region is Aw type, according to Köppen classification, with high annual rainfall and a well-defined dry period (Alvares *et al.*, 2014). Annual rainfall ranges from 1,420 to 2,160 mm, with an overall average of 1,450 mm during the rainy season (November to May) and 235 mm during the dry season (Silva *et al.*, 2018). The average annual temperature is 26°C, with the lowest values recorded in February and the highest in October (Moraes *et al.*, 2005).

Plants were collected in the rainy season - February 2018 and March 2019 - and in the dry season - June 2018 (Table 1). *Isoëtes cangae* specimens were collected from the southern portion of Amendoim pond, a permanent pond located in the ferruginous plateau south of the Serra dos Carajás (South Range) (Santos *et al.*, 2020). Amendoim pond has an area of 13.96 ha and a maximum depth of 7.8 m (Nobre *et al.*, 2019), with an average variation in the water column of 2.5 m over the year.

Isoetes serracarajensis specimens were collected from four temporarily flooded areas, two located on the ferruginous plateaus north of the Serra dos Carajás (North Range), N3 and N6; and two located on the South Range, S11

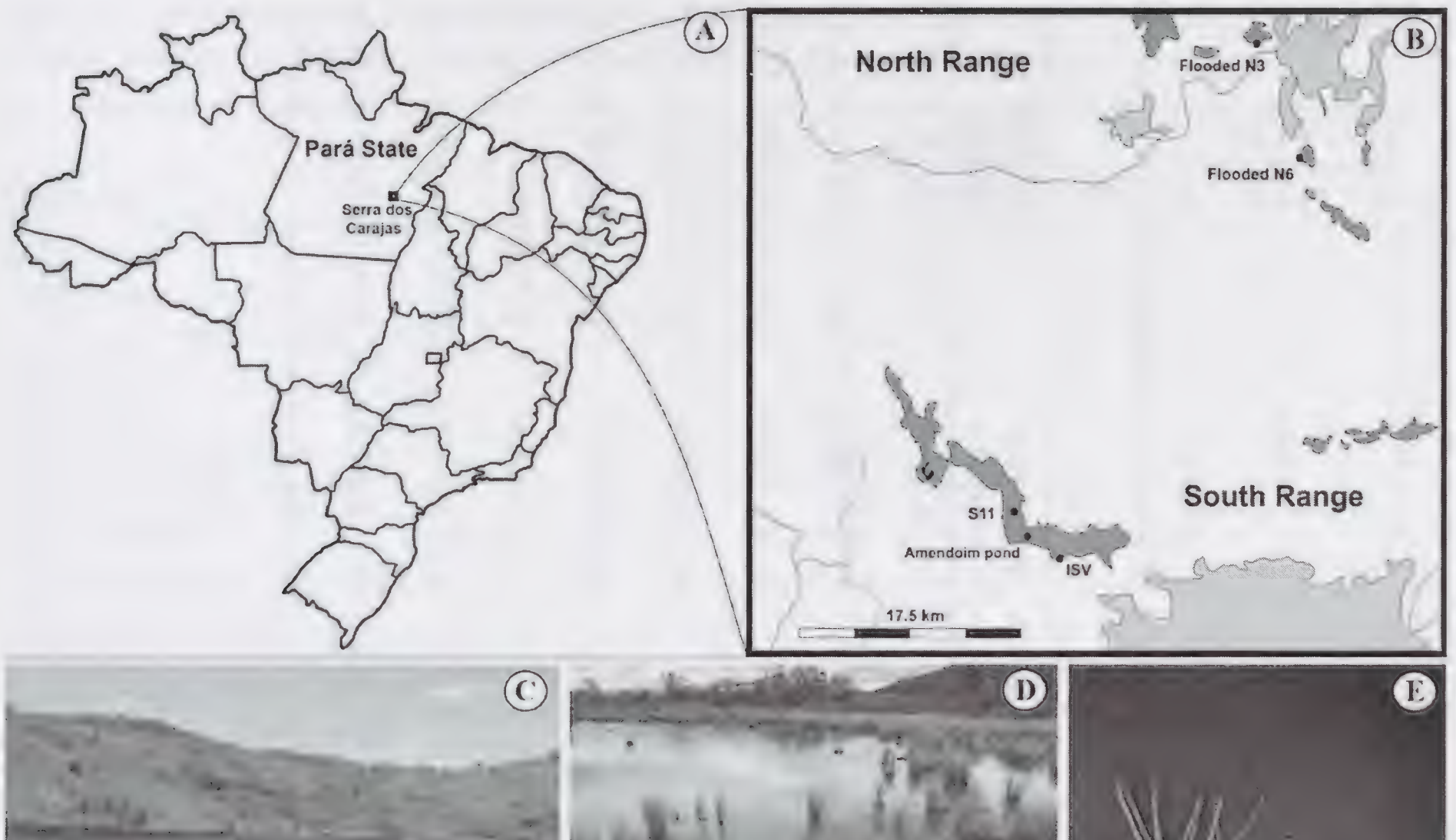


FIG. 1. Study area and specimens of *Isoëtes cangae* and *Isoëtes serracarajensis* sampled in the ferruginous outcrops of Serra dos Carajás, Pará State, Brazil. A. Brazil map B. Collection areas: *I. cangae* specimens were sampled in the Amendoim pond (South Range), and *I. serracarajensis* specimens were collected in the flooded areas N3 and N6 (North Range), S11 and ISV (South Range). C. Amendoim pond. D. Flooded area in S11. E. From left to right, specimen of *I. cangae* (*Campos et al.* 40, RFA) compared with specimens of *I. serracarajensis* collected from the flooded areas in ISV (*Campos et al.* 42, RFA), and S11 (*Campos et al.* 41, RFA). F-G. Flooded area in ISV. H. Specimens of *I. serracarajensis* collected in N3 (*Campos et al.* 44, RFA). I. Flooded area in N3. J. Flooded area in N6. K. Specimen of *I. serracarajensis* collected in N6 (*Campos et al.* 34, RFA). Dark grey areas indicate rocky outcrops and light grey human activity. Scale bars: E, H = 10 cm; K= 3cm.

TABLE 1. Specimens of *Isoëtes cangae* and *Isoëtes serracarajensis* collected in the North and South ranges of Serra dos Carajás (PA) over a year of sampling.

Species	Collection date	Locality	Coordinates	Water depth of plant site (m)	Accession number	Collector number
I. cangae	Feb/2018 (rainy season)	Serra Sul, Amendoim pond	6°24′03.73"S; 50°22′23.27"W	3.50	41149 (RFA)	*N.V. Campos et al. 36*
I. serracarajensis		Serra Norte, N6	6° 07′33.97"S; 50°10′39.43"W	0.40	41147 (RFA)	*N.V. Campos et al. 34*
I. cangae	Jun/2018 (dry season)	Serra Sul, Amendoim pond	6°24′03.73"S; 50°22′23.27"W	3.30	41153 (RFA)	*N.V. Campos et al. 40*
I. serracarajensis		Serra Sul, ISV	6° 24′31.39"S; 50°21′05.38"W	0.10	41155 (RFA)	*N.V. Campos et al. 42*
I. serracarajensis		Serra Sul, S11	6° 22′33.90"S; 50°23′00.34"W	0.10	41154 (RFA)	*N.V. Campos et al. 41*
I. cangae	Mar/2019 (rainy season)	Serra Sul, Amendoim pond	6°24′03.73"S; 50°22′23.27"W	4.60	41417 (RFA)	*N.V. Campos et al. 45*
I. serracarajensis		Serra Sul, S11	6° 22′33.90"S; 50°23′00.34"W	0.20	41416 (RFA)	*N.V. Campos et al. 44*
I. serracarajensis		Serra Norte, N3	6° 02′44.90"S; 50°12′34.68"W	0.80	41418 (RFA)	*N.V. Campos et al. 46*

TABLE 2. Morphometric and micromorphometric parameters measured in *Isoëtes cangae* and *I. serracarajensis* specimens collected from different localities and dates.

	February 2018		June 2018			March 2019		
Character	**IC**	**IS-N6**	**IC**	**IS-ISV**	**IS-S11**	**IC**	**IS-S11**	**IS-N3**
Leaf length	X	X	X	X	X	X	X	X
Leaf width			X	X	X	X	X	X
Leaf length/width ratio			X	X	X	X	X	X
Leaf number			X	X	X	X	X	X
Alae length			X	X	X	X	X	X
Alae/leaf length (%)			X	X	X	X	X	X
Velum coverage (%)			X	X	X	X	X	X
Velum length			X	X	X	X	X	X
Sporangium length	X	X	X	X				
Sporangium width	X	X	X	X				
Sporangium length/width ratio	X	X	X	X				
Spore number	X	X	X	X				
Number of sporangial cells	X	X	X	X				
Sporangial cell length	X	X	X	X				
Sporangial cell width	X	X	X	X				
Sporangial cell length/width ratio	X	X	X	X				
Polar axis	X	X	X	X				
Equatorial diameter	X	X	X	X				
Polar/Equatorial diameter	X	X	X	X				

IC: *Isoëtes cangae*, IS: *Isoëtes serracarajensis* (flooded areas N3 and N6 – North range; S11 and ISV – South range)

and ISV. Geographically, the shortest distance is between S11 and ISV populations, which are 5 km apart, and the greatest distance is between N3 and ISV, which are separated by about 45 km (Fig. 1). Populations N3 and N6 are 10 km apart. The flooded area in S11 has both the smallest area and depth, while the flooded areas in ISV and N3 have greater depth and a higher total water volume; the flooded area in N6 is extensive but shallow. The depth of the water bodies in the locality and at the time the plants were collected is shown in Table 1. It was not possible to collect adult specimens of all *I. serracarajensis* populations in both seasons due to the drought of some temporarily flooded areas and blocked access roads.

Fertile individuals of each *Isoëtes* population (n=12) were washed to remove excess substrate and transported to the Biodiversity and Sustainability Institute (NUPEM - UFRJ) for morphological and morphometric evaluations. Other specimens from each locality were pressed and dried in an oven at 70°C for the preparation of the vouchers, which were deposited at the Herbarium of the Federal University of Rio de Janeiro (RFA) (Table 1). Permission to collect the specimens was granted by the Chico Mendes Institute of Biodiversity of the Ministry of the Environment (ICMBio/MMA; numbers 641873 and 59724).

The parameters evaluated at each collection time/locality are described in Table 2. For each parameter analyzed, at least two different samples of *I. cangae* were considered, and at least one population of *I. serracarajensis* from the South Range and another from the North Range were used. The

comparisons were performed among species/populations collected at the same season or between different samples (dry and rainy seasons) from the same population. Means and standard deviations of all analyzed morphometric parameters from the vegetative and reproductive structures are shown in Table 3.

Morphology and morphometry of leaves.—Specimens of *I. cangae* and *I. serracarajensis* were photographed in their natural habitat and analyzed in the laboratory for morphological description. The specimens were observed with a stereomicroscope (Leica M205 FA, Mannheim, Germany) coupled to a digital camera (DFC550). The abaxial and adaxial leaf surfaces were peeled to examine stomata presence. Transverse sections of leaves were obtained to observe the number of air chambers. The histological samples were mounted on slides with glycerin water (50%) and viewed under an optical microscope (Olympus BX51, Tokyo, Japan) equipped with a digital camera (DP71). The same equipment was used in the morphological and micro-morphometric analyses of the reproductive structures.

The leaf morphometric analyses were performed using ten specimens from each population. Within each population, the specimens with the longest leaves and mature sporangia were measured. This standardization was done in order to choose individuals at the same stage of development. For each individual, the total leaf number was counted, and the maximum leaf length was measured. The means of leaf width and alae length were measured from three leaves of each specimen, and the ratio between the length and width of leaves, and the percentage of alae length over leaf length were calculated.

For the velum analysis, three sporangia per specimen were selected and photographed. Velum length was determined according to Budke, Hickey, and Heafner (2005). The measurements were obtained using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, USA). The percentage of velum coverage was calculated as the ratio between the exposed area of the sporangium adaxial surface and the total area of the sporangium covered by the velum. Velum area was measured using the Software Image J (Abràmoff, Magalhães, and Ram, 2004).

Morphology and morphometry of reproductive structures.—The same ten specimens selected for leaf measurements were used for analysis of reproductive structures. The shape and color of the sporangia and megaspores were examined and photographed under a stereomicroscope. The length and width of the megasporangia and the number of spores per megasporangium were evaluated in three mature megasporangia per specimen. The ratio between megasporangium length and width was calculated. Ten spores from each sporangium, totaling 300 megaspores per locality, were selected for micromorphometric analysis. Megaspores were photographed in equatorial view, and polar (P) and equatorial (E) diameters were measured using the Image-Pro Plus 4.5 Software (Media Cybernetics, Silver Spring, USA). The P/E ratio was calculated.

TABLE 3. Morphometric and micromorphometric parameters measured in *Isoëtes cangae* and *Isoëtes serracarajensis* specimens collected from different localities and dates.

	February 2018		June 2018			March 2019		
Character	**IC**	**IS-N6**	**IC**	**IS-ISV**	**IS-S11**	**IC**	**IS-S11**	**IS-N3**
Leaf length (cm)	28.21±2.80	12.03±1.69	38.17±3.36	12.33±0.89	7.46±0.86	31.50±4.85	6.49±0.55	36.41±1.27
Leaf width (cm)	-	-	1.68±0.24	1.77±0.15	1.24±0.13	1.60±0.14	1.50±0.20	1.32±0.15
Leaf length/width ratio	-	-	22.26±4.47	6.74±0.68	5.66±0.78	19.82±3.47	4.42±0.83	27.82±2.34
Leaf number	-	-	17.50±7.97	19.33±6.17	11.58±3.26	45.80±14.01	20.4±3.53	24.80±6.91
Alae length (cm)	-	-	3.69±0.65	1.74±0.49	1.26±0.22	4.91±0.74	1.50±0.35	2.25±0.48
Alae/leaf length (%)	-	-	10.08±1.22	14.75±4.19	18.18±2.68	16.13±4.43	23.37±6.31	6.18±1.29
Velum coverage (%)	-	-	21.82±5.36	9.99±3.32	14.43±5.49	23.23±3.14	8.34±2.13	10.89±2.36
Velum length (mm)	-	-	0.43±0.13	0.16±0.06	0.22±0.09	0.48±0.11	0.11±0.02	0.21±0.06
Megasporangium length (mm)	3.23±0.51	3.23±0.40	4.18±1.03	3.69±0.88	-	-	-	-
Megasporangium width (mm)	3.21±0.48	2.71±0.27	3.69±0.66	3.17±0.45	-	-	-	-
Megasporangium length/ width ratio	1.01±0.06	1.19±0.09	1.12±0.12	1.15±0.14	-	-	-	-
Spore number	54.23±30.98	58.67±16.52	98.53±44.56	41.00±22.65	-	-	-	-
Number of sporangial cells	70.71±7.74	51.38±7.30	71.91±8.33	48.27±7.08	-	-	-	-
Sporangial cell length (µm)	72.88±7.29	93.44±9.59	69.74±7.17	104.02±9.22	-	-	-	-
Sporangial cell width (µm)	19.98±1.69	22.41±1.93	19.81±1.36	22.79±2.75	-	-	-	-
Sporangial cell length/width ratio	3.67±0.41	4.18±0.44	3.53±0.37	4.60±0,49	-	-	-	-
Polar axis (µm)	520.56±20.98	499.14±17.97	519.50±22.14	572.95±28.32	-	-	-	-
Equatorial diameter (µm)	569.90±25.31	569.72±25.41	573.88±28.67	646.12±29.77	-	-	-	-
Polar/Equatorial diameters ratio	0.91±0.01	0.88±0.02	0.91±0.02	0.89±0.01	-	-	-	-

* Mean followed by standard deviation. IC: *Isoëtes cangae*, IS: *Isoëtes serracarajensis* (flooded areas in S11, ISV, N3 and N6, Serra dos Carajás, Pará State, Brazil)

After the removal of megaspores from the megasporangia, samples from the sporangial wall were separated, washed in distilled water, and affixed to glass slides (Budke, Hickey, and Heafner, 2005). The samples were mounted in 50% glycerin water, covered with a coverslip, and examined under an optical microscope. Three fields were photographed in each slide using a 40x objective lens (field area = 64.5 μm^2) to measure sporangial cell wall length and width and count the number of cells per field. Micromorphometric analyses were performed using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, USA). The ratio between sporangial cell length and width was calculated.

Micromorphology of megaspores.—Micromorphological characteristics of megaspores were measured from *I. cangae* and *I. serracarajensis* specimens collected from the flooded areas in the plateaus N3, N6, ISV, and S11, and stored in 70% alcohol. The megaspores were dried in silica gel, coated with gold (Quorum Q150RS Sputter Coater), and analyzed using a Scanning Electron Microscope (Zeiss SIGMA VP). Three specimens were selected from each population, and 10 megaspores from each specimen were photographed. Details of perispore micromorphology were observed in distal, proximal, and equatorial views. In this study, the terminology used to describe the spores is defined in Punt *et al.* (2007) and the classification of macro-structural elements of ornamentation in Pereira *et al.* (2016).

Statistical analysis.—Analysis of variance (ANOVA) was performed to test the differences in morphometric and micromorphometric parameters between species or populations studied or between the same population collected in different seasons (n=10). The Bartlett's test was used to test homogeneity of variance, and the Shapiro-Wilk's test was used to assess data normality. The Tukey's post-hoc test ($p < 0.05$) was used to compare means. The GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA) was used to analyze the data.

Results

Morphology of vegetative and reproductive structures.—*Isoëtes cangae* and *I. serracarajensis* leaves are recurved to erect and light green to olive-green (Fig. 1). *In vivo* leaves show a color gradient, from reddish-brown at the base of the leaf, close to the substrate, to light green in the most distal portion. Dark brown, persistent leaf bases enclose the corm in *I. cangae*. In cross-section of the median leaf portion, leaves of *I. cangae* and *I. serracarajensis* are quadrangular and semi-cylindrical, respectively, and show four air chambers (Fig. 2A-C). Stomata were observed in specimens of different populations of *I. serracarajensis*, but were absent in *I. cangae* (Fig. 2D-F).

Megasporangia of *Isoëtes cangae* and *I. serracarajensis* were elliptical to round. In *I. cangae,* megasporangia are light brown to dark brown (Fig. 2G-I), whereas megasporangia in *I. serracarajensis* vary from dark brown to reddish-

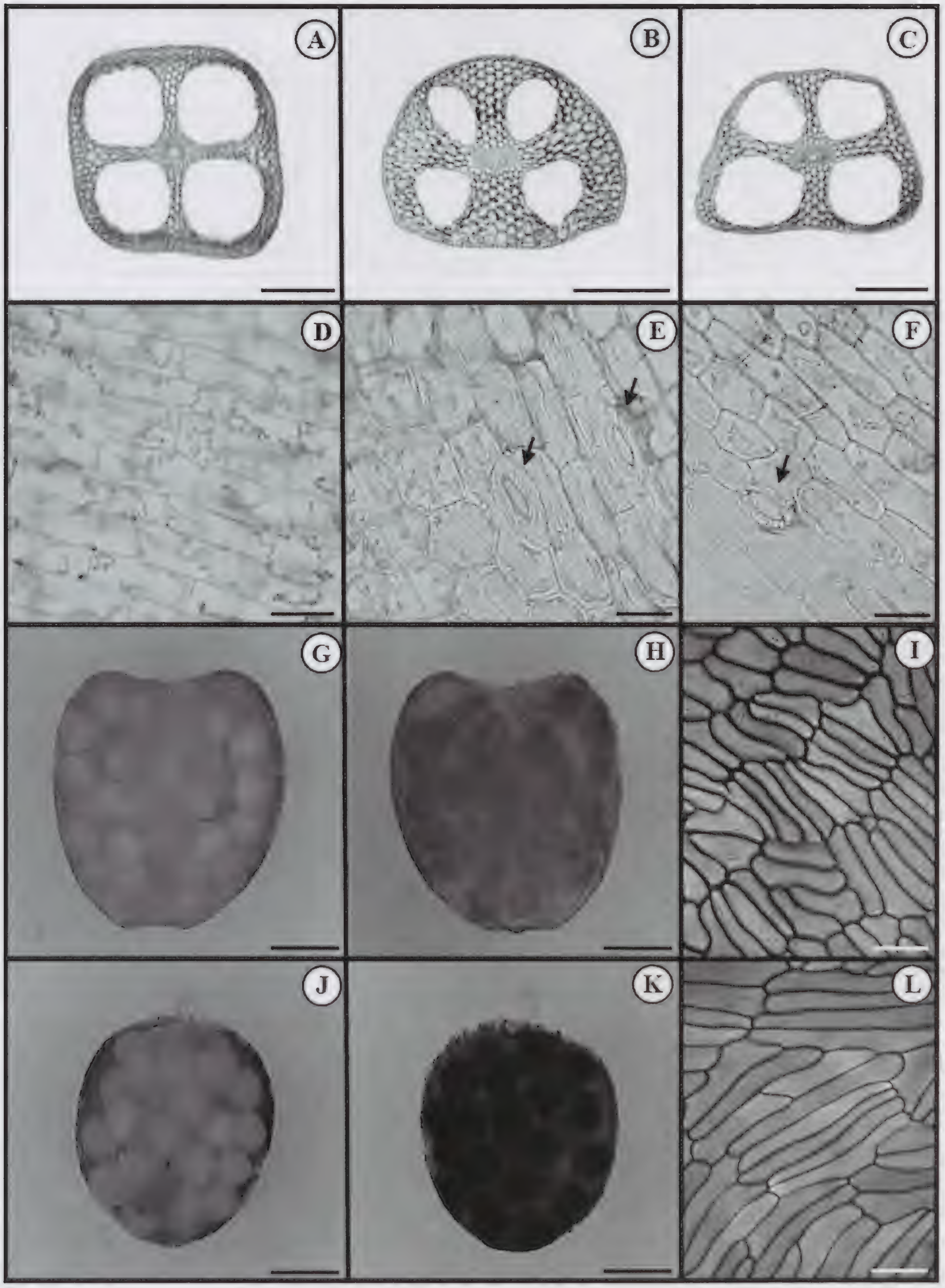


FIG. 2. Leaf cross-sections (A-C), epidermis (D-E), and megasporangia (D-I) of *Isoëtes* specimens collected in Serra dos Carajás-PA, Brazil (stereomicroscope). A-C. Leaf cross-sections of *Isoëtes cangae* (A; *Campos et al.* 45, RFA), and *Isoëtes serracarajensis* specimens from flooded areas in S11 (B; *Campos et al.* 46, RFA) and N3 (C; *Campos et al.* 44, RFA), showing the presence of four air chambers. D. Absence of stomata in *I. cangae* (*Campos et al.* 36, RFA). E-F. Anomocytic stomata in *I. serracarajensis* specimens from flooded areas in S11 (E; *Campos et al.* 41, RFA), and N3 (F; *Campos et al.* 44, RFA). G-I. Megasporangia of *I. cangae* (*Campos et al.* 36, RFA). J-L. Megasporangia of *I. serracarajensis* from the flooded area in N6 (*Campos et al.* 34, RFA). G and J. Abaxial view. H and K. Adaxial view. I and L. Detail of adaxial wall cells. Bars: A-C = 500 μm; D-F, I and L = 50 μm; G-H and J-K = 1 mm.

brown (Fig. 2J-L). In all samples, *I. cangae* and *I. serracarajensis* contained trilete, heteropolar, subspheroidal, and white to gray megaspores, with variation in size and color depending on the developmental stage. Spores of light color, in general, have a more pyramidal shape and are considered immature since the wall is still being formed. Mature spores are gray and have a rigid wall, with conspicuous macrostructural elements forming the perispore layer.

Megaspores of *I. cangae* collected in February and June 2018 showed no difference in the pattern of ornamentation, ranging from verrucate to tuberculate, with a predominance of round verrucae on the proximal surface and conical and pointed tubercles on the distal surface. Images of megaspores collected in June 2018 are shown in Fig. 3A-F. Laesures had straight and sloping sides, and a pyramidal shape (Fig. 3A). Apices were acute to obtuse (Fig. 3B, E).

Megaspores of specimens from the four populations of *I. serracarajensis* analyzed were predominantly verrucate, with rounded verrucae on the distal and proximal surfaces (Fig. 3G-L; 4A-L). Tubercles were also observed in some megaspores of specimens from the flooded area in ISV, with apices rounded to truncated (Fig. 3H-K). Laesures were broader than in *I. cangae*, with straight and parallel sides (Fig. 3G, 4A, D, G). The apex was obtuse and more rounded in populations from the flooded areas in ISV and N3 than in populations from S11 and N6 (Fig. 3H-K; 4B, E, H).

Morphometry of vegetative and reproductive structures.—Individuals of *Isoëtes cangae* and *Isoëtes serracarajensis* populations were collected from Serra dos Carajás (Pará, Brazil) during rainy and dry seasons for morphometric evaluations.

During the dry season – June 2018 – specimens of *I. cangae* and *I. serracarajensis* from the flooded areas in ISV and S11 were collected. The specimens did not differ in leaf number (Fig. 5A), while the leaf width was the greatest in *I. cangae* and *I. serracarajensis* – ISV (Fig. 5C). Leaf and alae length, and the leaf length/width ratio were higher in *I. cangae* than in *I. serracarajensis* populations (Fig. 5B, D-E), and the same relationship was observed for the percentage of coverage and length of the velum (Fig. 5G-H). The percentage of alae over leaf length was the lowest for *I. cangae*, although this species did not differ from *I. serracarajensis* – ISV (Fig. 5F). Megasporangium length and width, and the sporangium length/width ratio, did not differ between *I. cangae* and *I. serracarajensis* – ISV (Fig. 6A-C). The number of megaspores and the number of sporangial cells were the highest for *I. cangae* (Fig. 6D-E). The length and width of cells of the megasporangium wall (Fig. 6F-G), the sporangial wall cells length/width ratio (Fig. 6H), and the equatorial (E) and polar (P) diameters of megaspores (Fig. 7A-B) were the highest in *I. serracarajensis* –ISV. *Isoëtes cangae* specimens had higher P/E ratio than *I. serracarajensis* – ISV (Fig. 7C).

Two samples were obtained in the rainy season: February 2018 and March 2019. In the first one, specimens of *I. cangae* and *I. serracarajensis* from the

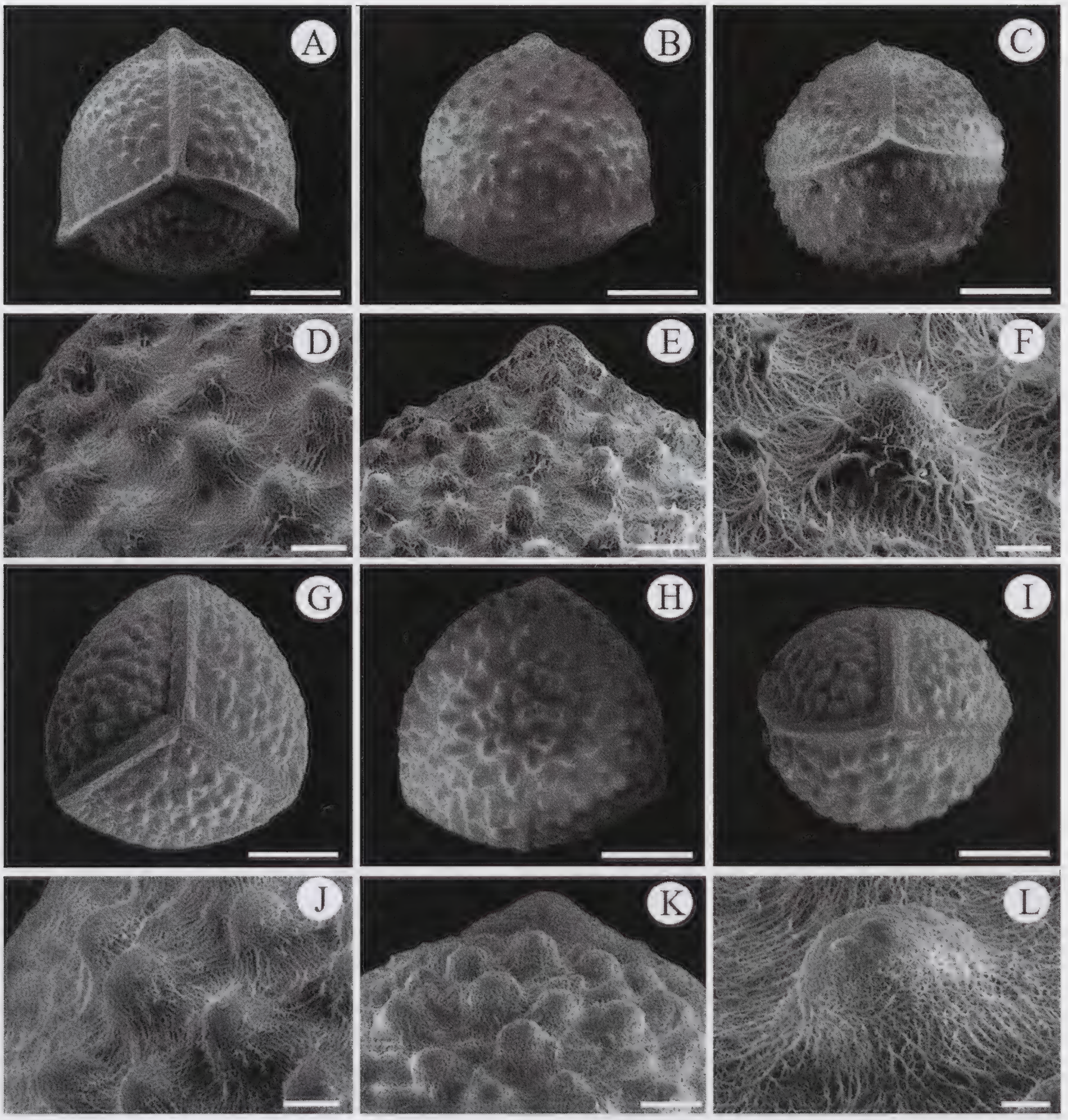


FIG. 3. Megaspores of *Isoëtes cangae* (A-F; *Campos et al.* 40, RFA) and *Isoëtes serracarajensis* from the flooded area in ISV (G-L; *Campos et al.* 42, RFA), Serra dos Carajás-PA, Brazil (Scanning Electron Microscopy). A and G. Proximal view. B and H. Distal view. C and I. Equatorial view. D and J. Detail of proximal view. E and K. Distal view showing apex of megaspore. F and L. Detail of macrostructural element: verruca. Bars: A-C; G-I = 200 μm; D = 20 μm; E, J-K = 50 μm; F = 10 μm; L = 15 μm.

flooded area N6 were collected. As observed in the dry season, leaf length was the greatest in *I. cangae* (Fig. 5B). The megasporangium length and width, and the number of megaspores did not differ between *I. cangae* and *I. serracarajensis* – N6 (Fig. 6A-B, D). However, the sporangium length/width ratio was greatest in *I. serracarajensis* – N6 (Fig. 6C). The same relationship observed in the dry season can be applied for the measurement of sporangial wall cells between species (Fig. 6E-G). However, differences were not observed

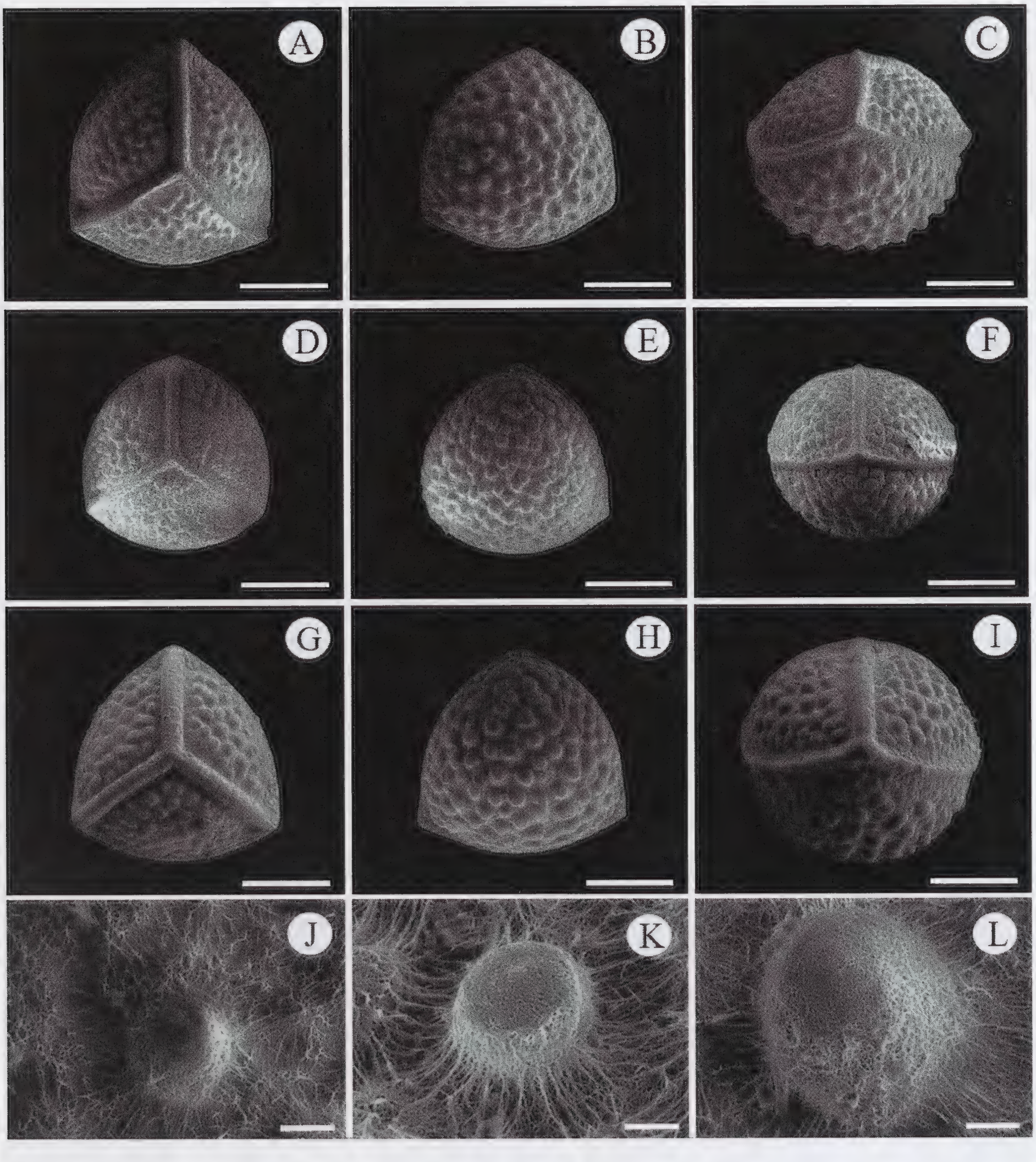


FIG. 4. Megaspores of *Isoëtes serracarajensis* from specimens collected in the flooded areas in S11 (A-C, J; *Campos et al.* 46, RFA), N3 (D-F, K; *Campos et al.* 44, RFA), and N6 (G-I, L; *Campos et al.* 34, RFA), Serra dos Carajás-PA, Brazil (Scanning Electron Microscopy). A, D and G. Proximal view. B, E and H. Distal view. C, F and I. Equatorial view. J-L. Detail of macrostructural element: verruca. Bars: A-I = 200 μm; J = 10 μm; K = 20 μm, L = 20 μm

between *I. cangae* and *I. serracarajensis* regarding the sporangial wall cells length/width ratio (Fig. 6H). The equatorial diameter (E) and polar axis (P) of megaspores did not differ between *I. cangae* and *I. serracarajensis* – N6 (Fig. 7A-B), and the P/E ratio was highest in *I. cangae* (Fig. 7C).

In the second sample, specimens of *I. cangae* and *I. serracarajensis* from the flooded areas S11 and N3 were collected. The leaf number, alae length, and the

FIG. 5. Leaf morphometry of specimens of *Isoëtes cangae* (black) and *Isoëtes serracarajensis* (gray) collected in February and June 2018 (rainy and dry season, respectively) and March 2019 (rainy season). A. Leaf number. B. Leaf length. C. Leaf width. D. Ratio between leaf length and leaf width. E. Alae length. F. Percentage of alae length over leaf length. G. Percentage of velum coverage. H. Velum length. IC = *I. cangae*; IS = *I. serracarajensis* (flooded areas in S11, ISV, and N3). Different letters above the columns indicate significant difference between the populations ($p<0.05$).

percentage of coverage and length of velum were higher in *I. cangae* than in *I. serracarajensis* populations (Fig. 5A, E, G-H). Leaf length and the leaf length/width ratio were the greatest in *I. serracarajensis* – N3 (Fig. 5B, D), and the percentage of alae over leaf length was the highest for *I. serracarajensis* – S11 (Fig. 5F). Leaf width was greater in *I. cangae* than in *I. serracarajensis* – N3 (Fig. 5C). The two *I. serracarajensis* populations differed in leaf and alae length, the leaf length/width ratio, and the percentage of alae over leaf length (Fig. 5B, D-F).

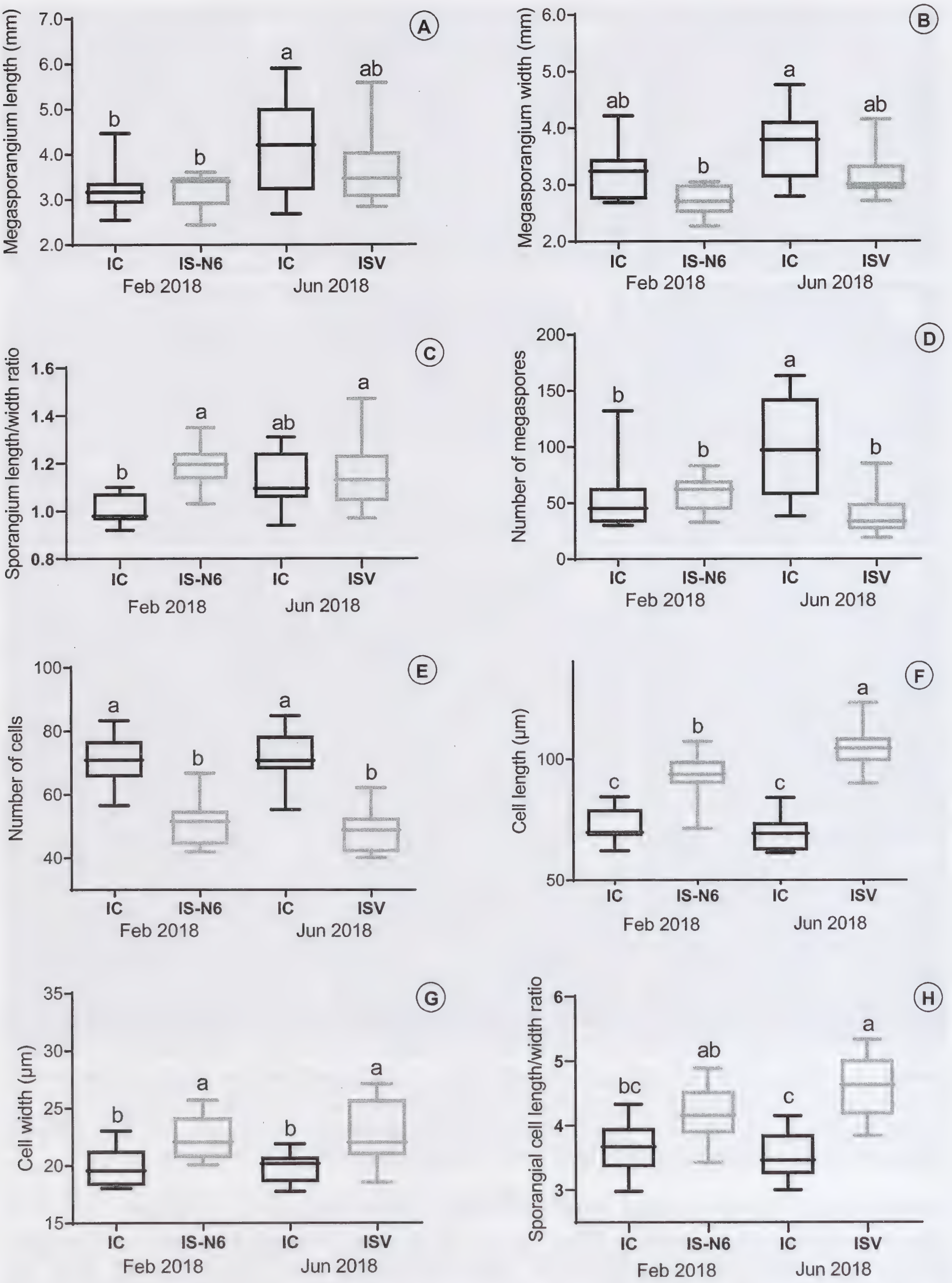
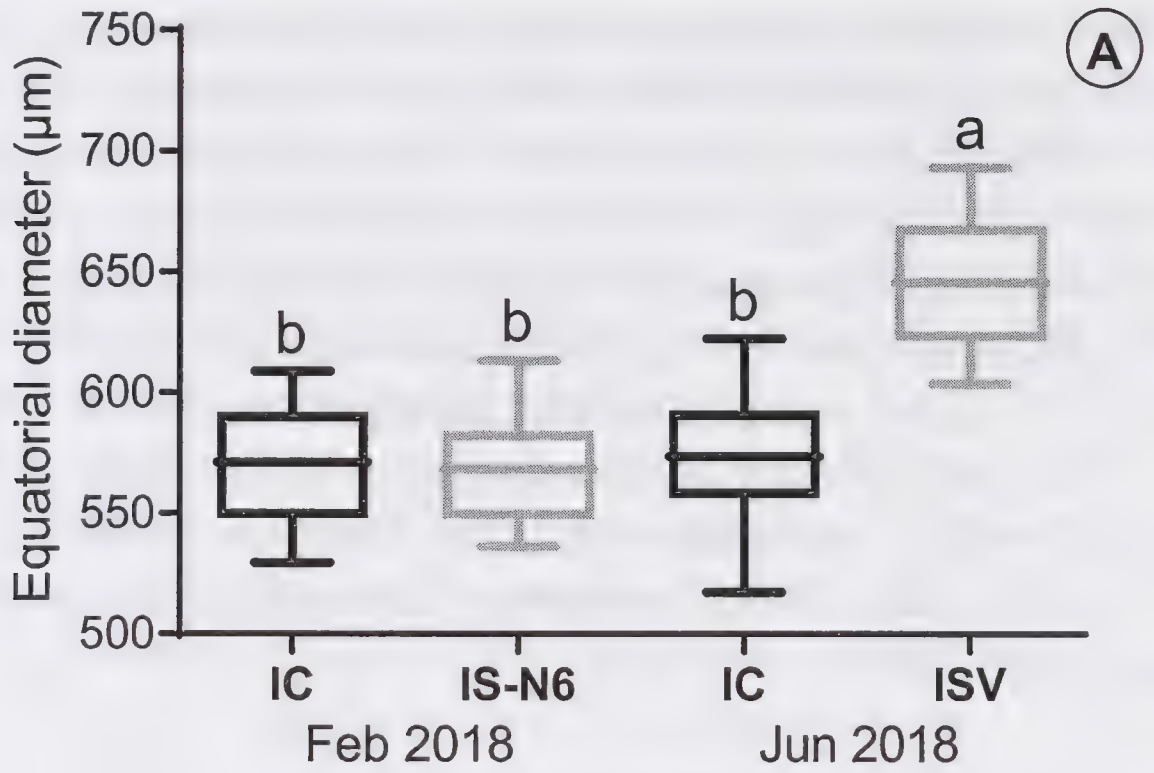
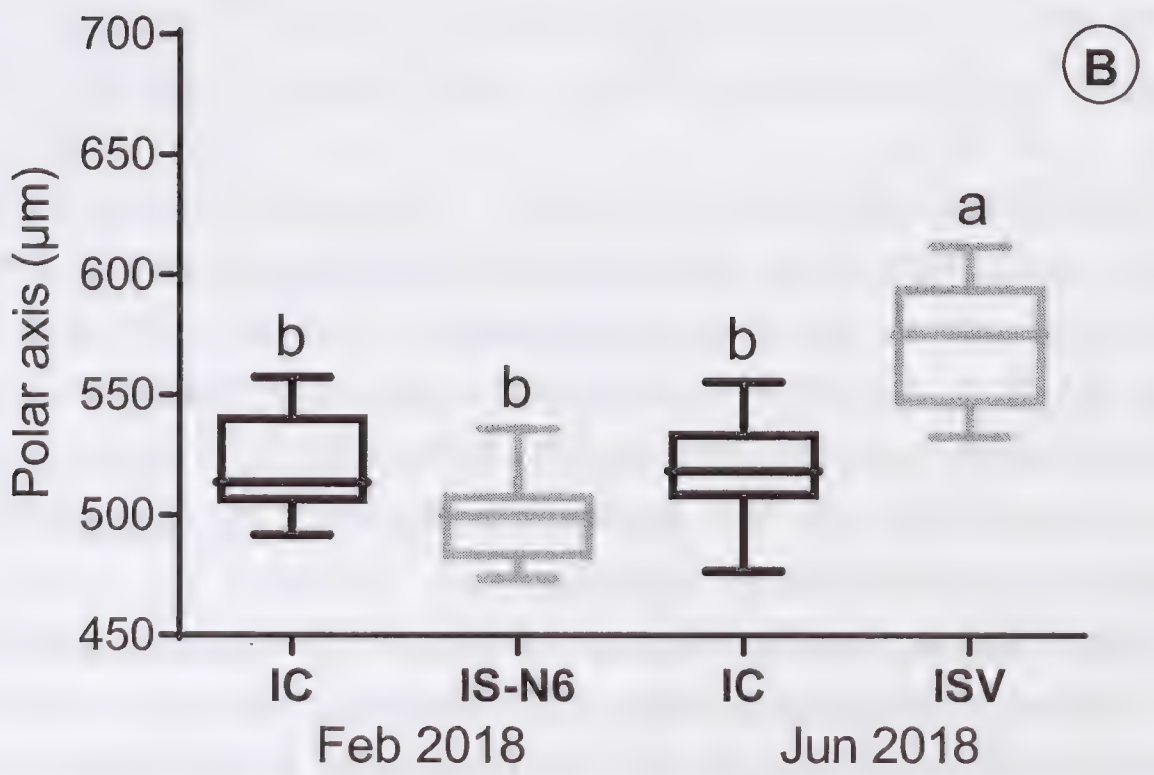


FIG. 6. Megasporangium morphometry of *Isoëtes cangae* (black) and *Isoëtes serracarajensis* (gray) specimens collected in February (rainy season) and June 2018 (dry season). A. Megasporangium length. B. Megasporangium width. C. Ratio between megasporangium length and width. D. Number of megaspores per megasporangium. E. Number of cells in the sporangial wall. F. Length of sporangial wall cells. G. Width of the sporangial wall cells. H. Ratio between length and width of the sporangial wall cells. IC = *I. cangae*; IS = *I. serracarajensis* (flooded areas in N6 and ISV). Different letters above the columns indicate significant difference between the populations ($p<0.05$).





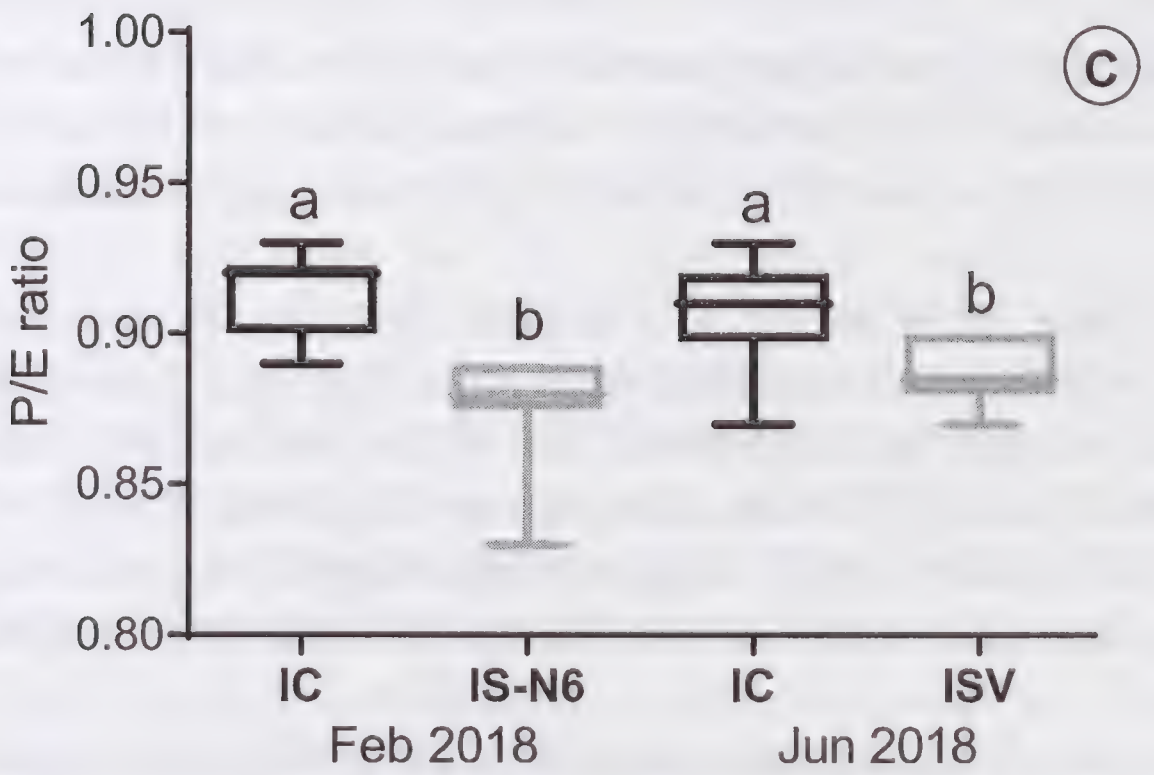


FIG. 7. Megaspore morphometry of *Isoëtes cangae* (black) and *Isoëtes serracarajensis* (gray) specimens collected in February (rainy season) and June 2018 (dry season). A. Equatorial diameter of megaspore. B. Polar axis of megaspore. C. Ratio of polar diameter to equatorial diameter of megaspore (P/E). IC = *I. cangae*; IS = *I. serracarajensis* (flooded areas in N6 and ISV). Different letters above the columns indicate significant difference between the populations ($p<0.05$).

Two populations were analyzed from both seasons: *I. cangae* and *I. serracarajensis* from the flooded area S11. The characters that did not show morphological variation in *I. cangae* over the seasons were: leaf width, leaf length/width ratio, percentage of alae over leaf length, percentage of velum coverage and velum length, megasporangium width, sporangium length/width ratio, sporangial wall cells, and megaspore measurements (Fig. 5C, D, G-H; Fig 6.B-C, E-H; Fig 7A-C). Leaf morphometric parameters did not differ between *I. serracarajensis* – S11 samplings, with exception of the leaf width (Fig. 5A-D). The percentage of velum coverage was the highest in the dry season in *I. serracarajensis* – S11 (Fig. 5G), however, the velum length did not differ between seasons (Fig. 5H).

Discussion

Isoëtes cangae and *Isoëtes serracarajensis* are endemic species from Serra dos Carajás that have been the subject of recent studies (Pereira *et al.*, 2016; Pereira, Arruda, and Salino, 2017; Nunes *et al.*, 2018; Caldeira *et al.*, 2019; Santos *et al.*, 2020; Zandonadi *et al.*, 2021). The former lives submerged at the bottom of a single pond at Serra Sul, while the latter is amphibious and occurs in different flooded areas spread over many plateaus in the Carajás region. Considering the high phenotypic plasticity and the morphological simplicity of the genus, this study was conducted to investigate the influence of seasonal and geographical variation on the morphology and morphometry of vegetative and reproductive organs of these species.

The quantitative characters that proved to be consistent for distinguishing *I. cangae* from *I. serracarajensis* were: alae length, velum length and coverage (%), morphometry of sporangial wall cells, and polar/equatorial megaspore diameter ratio (P/E). Qualitative characters were also useful for distinguishing species, including the presence of stomata, the color of megasporangia, and megaspore ornamentation. These results verify that the characters identified by previous studies were taxonomically relevant for characterizing *Isoëtes* species from Carajás (Pereira *et al.* 2016; Pereira, Arruda, and Salino, 2017; Nunes *et al.*, 2018).

Alae length was greater in *I. cangae* than in *I. serracarajensis*, even considering *I. serracarajensis*–N3 specimens, that had greater leaf length. In *I. cangae*, the alae length may represent 1/5 of the leaf while in *I. serracarajensis* it varies between 1/6-1/4 (Pereira, Arruda, and Salino, 2017). According to Kott and Britton (1985), alae length is generally related to leaf length; however, a direct relationship between these characters was not observed for these species.

In this study, velum coverage varied from 17–33% in *I. cangae* to 5–22% in *I. serracarajensis*. Our results, therefore, agreed with those of Pereira, Arruda, and Salino (2017) who found that the velum covers 1/4–1/5 and up to 1/5 of the sporangium surface in *I. cangae* and *I. serracarajensis*, respectively. Velum length in *I. cangae* was about double that of *I. serracarajensis*, although more discrete variation was observed by Pereira *et al.* (2016).

The color of the sporangia was related to sporangium maturation in both species, and was more reddish-brown in mature megasporangia of *I. serracarajensis*. Sporangial wall color was considered a distinguishing character between species (Kott and Britton, 1983; Pereira *et al.*, 2016). Further anatomical studies are necessary to clarify whether the sporangial pigmentation comes from the wall and/or the lumen of the epidermal cells (Budke, Hickey, and Heafner, 2005; Hall, 1971).

Megaspore ornamentation is considered one of the most stable characters in *Isoëtes* (Kott and Britton, 1985). As described by Pereira *et al.* (2016), the megaspores of *I. serracarajensis* are predominantly verrucate, whereas those of *I. cangae* are verrucate to tuberculate. However, tubercles were also observed in megaspores of some specimens of *I. serracarajensis* – ISV. According to Nunes *et al.* (2018), the megaspores of *I. serracarajensis* are verrucate, and rarely tuberculate. This variation may be explained by different populations sampled across studies. The P/E ratio was 0.87–0.93 and 0.83–0.90 in *I. cangae* and *I. serracarajensis*, respectively, and similar values were reported by Pereira *et al.* (2016).

The morphometry of sporangial wall cells and the presence of stomata are new characters with taxonomic relevance for these species. The presence or absence of stomata in *Isoëtes* has been used as a character to distinguish between species because they are present in both terrestrial and amphibious species and are absent in completely aquatic species (Pfeiffer, 1922). Stomata are probably formed in leaves exposed to air (emergent) during early development (Kott and Britton, 1985), and could be a variable character in amphibious species. The development of stomata in emerged leaves of amphibious *Isoëtes* species is related to the transition from the Crassulacean Acid Metabolism to the C3 metabolism (Raven *et al.*, 1988). The presence of stomata may be a reliable character, since both submerged and emerging leaves were considered in this study.

The number of cells per unit area of the megasporangium epidermis and the length and width of its cells proved to be stable characters with great taxonomic potential. According to Budke, Hickey, and Heafner (2005), analysis of cellular patterns on the sporangial epidermis can provide additional taxonomic characters, and it is important to consider a possible dimorphism between megasporangia and microsporangia.

Some characters that are usually used in describing *Isoëtes* species, such as the number of leaves, leaf length, sporangium size, number of megaspores, and megaspore diameter were found to be variable. The variation in leaf number of *I. cangae* can be related to leaf life span, since leaves senesce at the end of the rainy season, thus having a smaller number of leaves at the beginning of the dry season. Plant age also affects leaf number, which depends on stem development (Stokey, 1909), thus, considerable variation is expected among specimens. However, after the first year, plants tend to produce the same number of leaves each year (Karrfalt and Eggert, 1977). The lower leaf number of *I. serracarajensis* specimens, especially those from S11 collected in the dry season, may be related to the shorter life cycle of this species, once it inhabits

temporarily flooded areas. Plants that inhabit shallow waters may have a reduced leaf number due to the action of waves and other biotic and abiotic factors, while those in deep and calm waters have a larger leaf number, because older leaves may accumulate around the corm (Kott and Britton, 1985). The leaf number per plant in *I. serracarajensis* must be carefully examined since the species propagates vegetatively by producing new plants from the leaf base of juvenile specimens (Pereira, Arruda, and Salino, 2017).

The highest leaf length observed in *I. cangae* specimens collected in the dry season, at the time that the water level in the pond was reduced, suggests a strategy to compensate for increases in competition for light. The high plasticity in shoot elongation confers an advantage in environments where competition for light is a significant selection factor (Schmitt, McCormac, and Smith, 1995). The greatest leaf length of *I. serracarajensis*–N3 in the rainy season may be associated with the high water column of this flooded area that favor leaf elongation, when compared to other *I. serracarajensis* populations, (Gacia and Ballesteros, 1993). In the dry season, N3 was completely dry and only a few individuals of *I. serracarajensis* were visually observed growing in the middle of the groundcover vegetation, with one or two long leaves (infertile specimens that were not analyzed). This suggests that *I. serracarajensis* is tolerant to desiccation, as reported in *I. luetzelburgii* U.Weber (Pereira, Silvestre, and Santiago, 2018). Specimens of *I. serracarajensis* –S11 showed short leaves in both seasons, although this flooded area did not present much variation in water level. The rainfall regime and the characteristics of each habitat, in association with genetic diversity, seem to regulate growth in *I. serracarajensis*.

The seasonal variation observed for megasporangium size and the number of megaspores per sporangium in *I. cangae* indicates that reproductive structures can undergo changes depending on age and environmental conditions. According to Budke, Hickey, and Heafner (2005), the size and the number of megaspores per sporangium in *I. andicola* (Amstutz) L. D. Gómez are difficult characters to use in taxonomic studies because plants on their first reproductive cycles produce smaller sporangia with fewer spores. Interestingly, *I. serracarajensis*–ISV and *I. serracarajensis*–N6 did not differ in megasporangium size and megaspores number; however, specimens collected in the flooded area ISV presented megaspores with higher diameters. These differences may be explained by both genetic and/or ecological factors. Santos *et al.* (2020) investigated the genetic diversity of the same *I. serracarajensis* populations and detected genetic differentiation among the analyzed populations of 45%. Also, they have found that these populations have low gene flow. Temporarily flooded areas play an important role in isolating different populations and, therefore, in the evolution of species (Troia, Oddo, and Bellini, 2003). Thus, the phenotypic plasticity of *I. serracarajensis* might have been important for its adaptation to varying environmental conditions, culminating in a broader distribution throughout the ferruginous mountain ranges of Pará.

Finally, it is well known that morphological characters of *Isoëtes* are highly plastic and reflect the environmental conditions where the plants grow (Kott and Britton, 1985). Despite this plasticity, variation in plant morphology and morphometry can be measured and quantified, and such analyses can provide additional biological and taxonomic information (Budke, Hickey, and Heafner, 2005). The consideration of multiple characters is necessary to establish the limits and affinities between the species of *Isoëtes* (Hickey, 1986; Pereira, Arruda, and Salino, 2017).

In this study, the vegetative and reproductive traits of *Isoëtes* species were characterized as having low or high variability, considering intraspecific and seasonal characteristics. These features should be carefully defined when undertaking seasonal and interpopulation studies, taking into account the morphology, ecology, and cytogenetics of the taxa. The current findings add further information on the biology of the species, and reveal additional characters with taxonomic importance. Considering these conditions is of critical importance when developing effective conservation plans for *Isoëtes* in Carajás.

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Literature Cited

Abràmoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with Image J. Biophotonics International 11:36–42.

Alvares, C. A., J. L. Stape, P. C. Sentelhas, J. L. M. Gonçalves, and G. Sparovek. 2014. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22:711–728.

Barrett, S. C. H., C. G. Eckert, and B. C. Husband. 1993. Evolutionary processes in aquatic plants. Aquatic Botany 44:105–145.

Budke, J. M., R. J. Hickey, and K. D. Heafner. 2005. Analysis of morphological and anatomical characteristics of *Isoëtes* using *Isoëtes tennesseensis*. Brittonia 57:167–182.

Caldeira, C. F., C. B. Abranches, M. Gastauer, S. J. Ramos, J. T. F. Guimarães, J. B. S. Pereira, and J. O. Siqueira. 2019. Sporeling regeneration and *ex situ* growth of *Isoëtes cangae* (Isoetaceae): Initial steps towards the conservation of a rare Amazonian quillwort. Aquatic Botany 152:51–58.

Clevering, O. A. and M. P. J. Hundsheid. 1998. Plastic and non-plastic variation in growth of newly established clones of *Scirpus* (*Bolboschoemus*) *maritimus* L. grown at different water depth. Aquatic Botany 62:1–17.

Dorken, M. E. and S. C. H. Barret. 2004. Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. Journal of Ecology 92:32–44.

Gacia, E. and E. Ballesteros. 1993. Population and individual variability of *Isoetes lacustris* L. with depth in a Pyrenean lake. Aquatic Botany 46:35–47.

Giulietti, A. M., T. C. Giannini, N. F. O. Mota, M. T. C. Watanabe, P. L. Viana, M. Pastore, U. C. S. Silva, M. F. Siqueira, J. R. Pirani, H. C. Lima, J. B. S. Pereira, R. M. Brito, *et al.* 2019. Edaphic endemism in the Amazon: Vascular plants of the canga of Carajás, Brazil. Botanical Review 24:357–383.

Guimarães, J. T. F., P. K. Sahoo, P. W. M. Souza-Filho, M. M. J. C. de Figueiredo, L. S. Reis, M. S. da Silva, and T. M. Rodrigues. 2019. Holocene history of a lake filling and vegetation dynamics of the Serra Sul dos Carajás, southeast Amazonia. Anais da Academia Brasileira de Ciências 91(Suppl. 2):e20160916.

Hall, J. B. 1971. Observations on *Isoëtes* in Ghana. Botanical Journal of the Linnean Society 64:117–139.

Hickey, R. J. 1986. The early evolutionary and morphological diversity of *Isoëtes*, with descriptions of two new Neotropical species. Systematic Botany 11:309–321.

Karrfalt, E. E., and D. A. Eggert. 1977. The comparative morphology and development of *Isoetes* L. II. Branching of the base of the corm in *I. tuckermanii* A. Br. and *I. nuttallii* A. Br. Botanical Gazette 138:357–368.

Kott, L. S., and D. M. Britton. 1983. Spore morphology and taxonomy of *Isoëtes* in northeastern North America. Canadian Journal of Botany 61:3140–3163.

Kott, L. S., and D. M. Britton. 1985. Role of morphological characteristics of leaves and the sporangial region in the taxonomy of *Isoëtes* in Northeastern North America. American Fern Journal 75:44–55.

Liu, H., Q. F. Wang, and W. C. Taylor. 2006. Morphological and anatomical variation in sporophylls of *Isoëtes sinensis* Palmer (Isoetaceae), an endangered quillwort in China. American Fern Journal 96:67–75.

Marsden, C. R. 1976. A new subspecies of *Isoëtes coromandelina* from northern Australia. Contributions from Herbarium Australiense 24:1–10.

Maurity, C. W. and B. Kotschoubey. 1995. Evolução recente da cobertura de alteração no Platô N1-Serra dos Carajás-PA. Degradação, pseudocarstificação, espeleotemas. Boletim do Museu Paraense Emílio Goeldi, Série Ciências da Terra 7:331–362.

Moraes, B. C., J. M. N. Costa, A. C. L. M. Costa, and M. H. Costa. 2005. Variação espacial e temporal da precipitação no estado do Pará. Acta Amazonica 35:207–214.

Nobre, R. L. G., A. Caliman, R. D. Guariento, R. L. Bozelli, and L. S. Carneiro. 2019. Efeitos da introdução de um peixe onívoro sobre a biodiversidade e funcionamento de um lago de altitude amazônico. Acta Amazonica 49:221–231.

Nunes, G. L., R. R. M. Oliveira, J. T. F. Guimarães, A. M. Giulietti, C. Caldeira, S. Vasconcelos, E. Pires, M. Dias, M. Watanabe, J. Pereira, R. Jaffé, C. H. M. M. Bandeira, *et al.* 2018. Quillworts from the Amazon: A multidisciplinary populational study on *Isoëtes serracarajensis* and *Isoëtes cangae*. PloS one 13:p.e0201417.

Page, C. N. 2002. Ecological strategies in fern evolution: a neopteridological overview. Review of Palaeobotany and Palynology 119:1–33.

Pereira, J. B. S. and P. H. Labiak. 2013. A new species of *Isoëtes* with tuberculate spores from Southeastern Brazil (Isoetaceae). Systematic Botany 38:869–874.

Pereira, J. B. S., A. Salino, A. Arruda, and T. Stützel. 2016. Two new species of *Isoëtes* (Isoetaceae) from northern Brazil. Phytotaxa 2:141–148.

Pereira, J. B. S., A. J. Arruda, and A. Salino. 2017. Flora of the cangas of Serra dos Carajás, Pará, Brazil: Isoetaceae. Rodriguésia 68:853–857.

Pereira, J. B. S., T. Stützel, and C. Schulz. 2017. *Isoetes nana*, a new species from the coastal mountains of southeastern Brazil. PhytoKeys 89:91.

Pereira, J. B. S., L. C. Silvestre, and A. C. P. Santiago. 2018. Lectotypification and observations on the morphology, distribution and conservation status of *Isoetes luetzelburgii* (Isoetaceae). Phytotaxa 364:289–295.

Pereira, J. B. S, J. T. F. Guimarães, and M. T. Watanabe. 2019. *Isoetes dubsii* and *Isoetes santacruzensis*, two new species from lowland areas in South America. PhytoKeys 131:57–67.

Pfeiffer, N. E. 1922. Monograph of the Isoetaceae. Annals of the Missouri Botanical Garden 9:79–232.

Punt, W., P. P. Hoen, S. Blackmore, S. Nilsson, and A. Le Thomas. 2007. Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143:1–81.

Raven, J. A., L. L. Handley, J. J. MacFarlane, S. McInroy, L. McKenzie, and J. H. Richards. 1988. The role of root CO_2 uptake and CAM in inorganic C acquisition by plants of the isoetid life form. A review, with new data on *Eriocaulon decangulare*. New Phytologist 108:125–148.

Santos, M. P., J. V. R. Araujo, A. V. S. A Lopes, J. C. F. Vettorazzi, R. N. da Fonseca, E. N. Calderón, M. S. B. Boechat, F. A. Santana Arêdes, M. G. Pereira, N. V. Campos, F. M. G. dos Santos, T. N. Fernandes, *et al.* 2020. The genetic diversity and population structure of two endemic Amazonian quillwort (*Isoëtes* L.) species. PeerJ 8:1–15.

Schmitt, J., A. C. McCormac, and H. Smith. 1995. A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. American Naturalist 146:937–953.

Sharpe, J. M., K. Mehltreter, and L. R. Walker. 2010. Ecological importance of ferns. Pp.1-18, *in* K. Mehltreter, L. R. Walker, and J. M. Sharpe (eds.) *Fern Ecology*. Cambridge University Press, New York.

Silva, M. S., J. T. F. Guimarães, P. W. M. Souza Filho, W. N. Júnior, P. K. Sahoo, F. R. da Costa, R. O. S. Júnior, T.M. Rodrigues, and M. F. da Costa. 2018. Morphology and morphometry of upland lakes over lateritic crust, Serra dos Carajás, southeastern Amazon region. Anais da Academia Brasileira de Ciências 90:1309–1325.

Stokey, A. G. 1909. The anatomy of *Isoetes*. Botanical Gazette 47:311–335.

Sultan, S. E. 2000. Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5:537–542.

Takamiya, M., M. Watanabe, and K. Ono. 1997. Biosystematic studies on the genus *Isoëtes* (Isoetaceae) in Japan. IV. Morphology and anatomy of sporophytes, phytogeography, and taxonomy. Acta Phytotaxonomica et Geobotanica 48:89–122.

Taylor, W. C. and R. J. Hickey. 1992. Habitat, evolution, and speciation in *Isoëtes*. Annals of the Missouri Botanical Garden 79:613-–622.

Troia, A., E. Oddo, and E. Bellini. 2003. Morphological observations on the co-occurring geophytes *Isoëtes duriei* Bory (Lycophyta, Isoëtaceae) and *Romulea columnae* Sebast. Et Mauri (Anthophyta, Iridaceae). Pp. 185–189, *in* C. Subhash and S. Mrittunjai (eds). *Pteridology in the New Millennium*. Springer, Dordrecht.

Troia, A., J. B. Pereira, C. Kim, and W. C. Taylor. 2016. The genus *Isoetes* (Isoetaceae): a provisional checklist of the accepted and unresolved taxa. Phytotaxa 277:101–145.

Viana, P. L., N. F. O. Mota, A. S. B. Gil, A. Salino, D. C. Zappi, R. M. Harley, A. L. Ilkiu-Borges, R. de S. Secco, T. E. Almeida, M. T. C. Watanabe, J. U. M. dos Santos, M. Trovó, *et al.* 2016. Flora of the cangas of the Serra dos Carajás, Pará, Brazil: history, study area and methodology. Rodriguésia 67:1107–1124.

Vöge, M. 2003. Environmentally related demography: Field studies on *Isoetes lacustris* L. (Lycophyta, Isoetaceae) in Europe. Pp. 233–260, *in*: S. Chandra and M. Srivastava (eds.) *Pteridology in the New Millennium*. Springer, Dordrecht.

Wells, C. L. and M. Pigliucci. 2000. Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. Perspectives in Plant Ecology, Evolution and Systematics 3:1–18.

Yadav, B. B., S. K. Singh, M. Srivastava, and G. K. Srivastava. 2012. Comparative studies of six populations of *Isoëtes panchganiensis* from India. Turkish Journal of Botany 36:667–676.

Zandonadi, D. B., H. M. Duarte, M. P. Santos, L. A. S. Prado, R. L. Martins, E. N. Calderon, A. C. A. Fernandes, Q. S. Santos, F. J. G. Nunes, L. C. F. Ribeiro, T. N. Fernandes, A. Castilho, *et al.* 2021. Ecophysiology of two endemic Amazon quillworts. Aquatic Botany 170:103350.

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First Record of Sporangiasters in the Blechnaceae

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Abstract.—Sporangiasters are an uncommon type of paraphyses, occurring in a handful of leptosporangiate fern genera. We report sporangiasters in *Parablechnum nesophilum,* which is their first recorded occurrence in the Blechnaceae. We provide evidence of their homology to sporangia and discuss their functional significance. The presence of sporangiasters in *P. nesophilum* is surprising because the species has a well-developed indusium, and paraphyses are generally most common in exindusiate ferns. We surmise that these sporangiasters act as a second stage of protection for the developing sporangia after the indusium retracts. Alternatively, it is possible that sporangiasters may influence the vagility of spores by inhibiting dehiscence and limiting the ability of the annulus to disperse its spores.

Key Words.—Anatomy, morphology, paraphyses, sori, spore dispersal

Paraphyses of ferns are sterile structures that occur mixed among sporangia (Wagner, 1964; Parris, 1997). They may be borne either on the receptacle or on the sporangia themselves, such as on the sporangial stalks or capsules. Opinions have varied concerning whether sporangium-borne paraphyses should be considered paraphyses or given an alternate term, and whether the term paraphyses should also pertain to hairs that are like those elsewhere on the lamina (A. F. Tryon, 1965; R. Tryon, 1965; Wagner, 1964; 1965). In either case, the functional significance of paraphyses is thought be of protection for the developing sporangia against desiccation, herbivory, or from each other as they grow in size (Wagner, 1964).

Paraphyses are widespread in leptosporangiate ferns (Copeland, 1947; Wilson, 1959), but they are unevenly distributed. They are common and diagnostic in groups such as *Pteris* (Bower, 1928), the Vittarioideae genera (Schuettpeltz *et al.*, 2016) and the Elaphoglossoideae (Moran, Labiak, and Sundue, 2010), where they occur upon the receptacle, and in many genera of Thelypteridaceae (Smith, 1990), Polypodiaceae (Baayen and Hennipman, 1987; Sundue, Islam, and Ranker, 2010) where they are prominent upon sporangial stalks and capsules. However, in other large groups such as Lindsaeaceae (Lehtonen *et al.*, 2010), Dennstaedtiaceae (Schwartsburd *et al.*, 2020), Aspleniaceae (Sundue and Rothfels, 2014), Athyriaceae (Wei *et al.*, 2018), Dryopteridoideae (Zhang *et al.*, 2013) and Tectariaceae (Holttum, 1991)

are absent or rare. In some instances, they are similar or identical to leaf indument. In other cases, they appear to be modified sporangia and have been termed sporangiasters (the suffix *-aster* indicating "incomplete likeness"; Stearn, 1992). Sporangiasters are less common than other types of paraphyses and occur only in a handful of leptosporangiate fern genera. They are best known in *Polypodium*, where they develop a typical sporangial stalk, but instead of forming a spore-bearing capsule, the apex develops into a clear vitreous sphere adorned with bulbous protrusions (Martens and Pirard, 1943). They develop precociously, well before the sporangia mature, and their rounded and enlarged apices form a roof over the young sporangia. When the sporangia mature, their stalks elongate, pushing the capsules above the sporangiasters, enabling subsequent dehiscence (Peterson and Kott, 1979).

Here, we report the occurrence of sporangiasters in *Parablechnum nesophilum* (T.C. Chambers & P.A. Farrant) Gasper & Salino. This is the first report of the occurrence of sporangiasters in the Blechnaceae. We provide evidence of their homology to sporangia and discuss their functional significance.

Materials and methods

This study is based on specimens of *Parablechnum nesophilum* (Fig. 1 A–E) collected in East New Britain, Papua New Guinea (*Sundue 4251*, BISH, LAE, MACB, VT). The species known primarily from the small islands of New Britain, New Ireland, and the Solomon Islands, and Santa Cruz Islands, but has also been recorded from mainland Papua New Guinea (Chambers, Edwards, and Johns, 2005; Chen *et al.*, 2017; Cámara-Leret *et al.*, 2020). The binomial means "island-loving."

Plant microscopy protocols followed Ruzin (1999), and 1% TBO (Toludine O Blue) and fluoroglycine were chosen for staining, which have been widely used in anatomical studies in ferns (*e.g.*, Prada *et al.*, 2016). The observations with light microscopy were taken with a Nikon Labophot-2 microscope with a Coolpix MDC camera. The same specimen was observed using scanning electron microscopy (SEM). The samples were mounted on a sample holder with carbon adhesive. Before observation, each sample was coated with a gold layer and observed on a JSM 6400 JEOL scanning electron microscope operating at 20 kv. The observations were made at the National Center for Electron Microscopy (CNME) of the Complutense University of Madrid, Spain. The nomenclature used for the sporangium characters is based on the combination of Lellinger (2002) and Wilson (1959) presented in Molino *et al.* (2020).

Results

The sori of *Parablechnum nesophilum* are linear and occur on the lamina abaxially along both sides of the costa, forming a coenosorus. They are covered by continuous dark brown membranaceous indusia, formed by the recurved

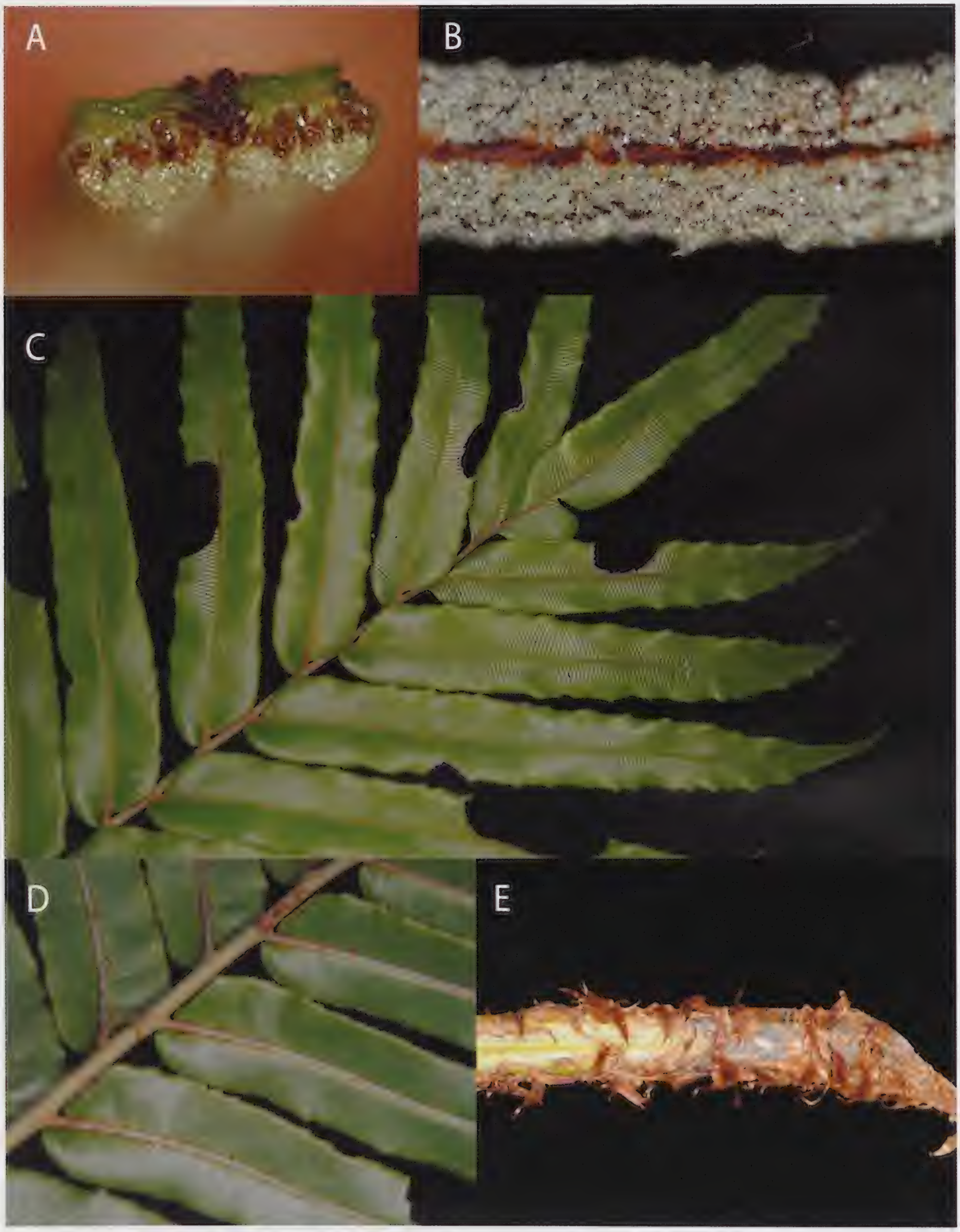

FIG. 1. *Parablechnum nesophilum* (*Sundue 4251*). A. Cross section of fertile pinna showing vitreous sporangiasters above brown sporangia. B. Abaxial fertile pinna showing vitreous sporangiasters. C. Adaxial surface of sterile lamina. D. Abaxial surface of sterile lamina. E. Petiole with scales.

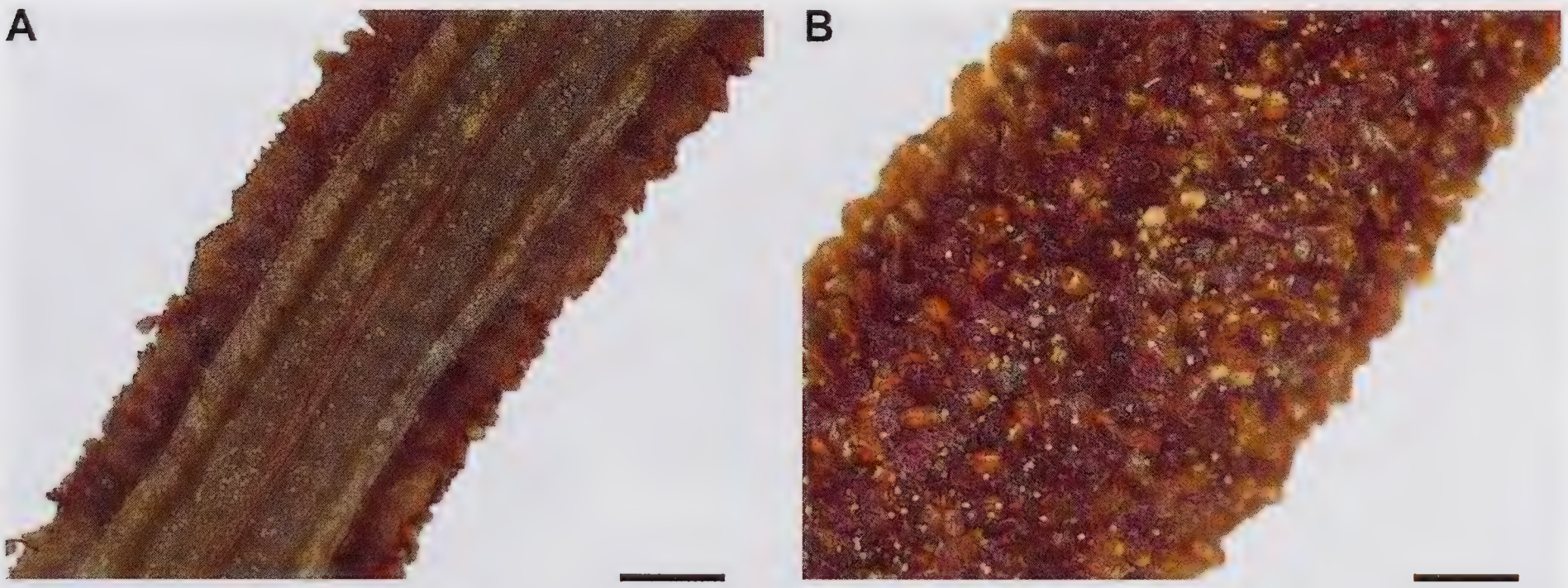

FIG. 2. Pinnae of *P. nesophilum* (*Sundue 4251*). A. Adaxial side showing remains of retracted indusium; B. Abaxial side, showing sporangia and sporangiasters concolorous after drying. Scalebar = 1.5 mm in A; 1 mm in B.

margin of the pinna. As in all Blechnaceae, the indusia open towards the costa (Fig. 2).

Pinna anatomy.—As in other *Parablechnum*, the receptacle is elongate, covering most of the abaxial surface of the pinnae. The costa is grooved adaxially and prominent abaxially, with sclerotic cells in the grove and the keel, which stain blue with TBO and bright pink with fluoroglycine (Fig 3. A, B). Three vascular bundles were visible in the costa; a small central bundle, and two larger lateral bundles. The largest ones were provided with an interrupted dark circumdermal sheath towards the abaxial side of the costa. This band remains black after the staining with both TBO and fluoroglycine (Fig. 3B). The indusium is composed of several layers of cells (Figs. 3A, 3C).

Sporangia.—Sporangia were thin-walled with a vertical annulus interrupted at the stalk, which is three cells wide, similar to most other Polypodiales (Fig. 3). Sporangia in various stages of development were present in the studied material (Fig. 3C). Fully developed sporangia had an annulus with 20–24 cells and capsules 269.4 ± 15.8 μm wide and 410.2 ± 33.9 μm long, with three (rarely five) lip cells, one above and two below (rarely two above and three below). The upper and lower lips were 39.6 ± 22.7 μm and 47.5 ± 0.2 μm, respectively. In most cases, four epistomial cells and one hypostomial cells are present (rarely three and two cells, respectively). The pedicel is 311.6 ± 125.6 μm long, with a three-cell rosette 64.4 ± 15.9 μm long.

Sporangiasters.—These occurred among the spore-bearing sporangia, and were 1.3 ± 0.2 μm long. Their stalks were identical to those of the sporangia, but their apices that developed into vitreous clavate heads of 6–10 subglobose cells (Figs. 3E, 4). Unlike spore-bearing capsules, the clavate heads lacked a central cavity and showed no signs of differentiation into an annulus or stomium. The sporangiasters occur at roughly the same density as the regular sporangia and are ca. 1.5x taller than the sporangia.

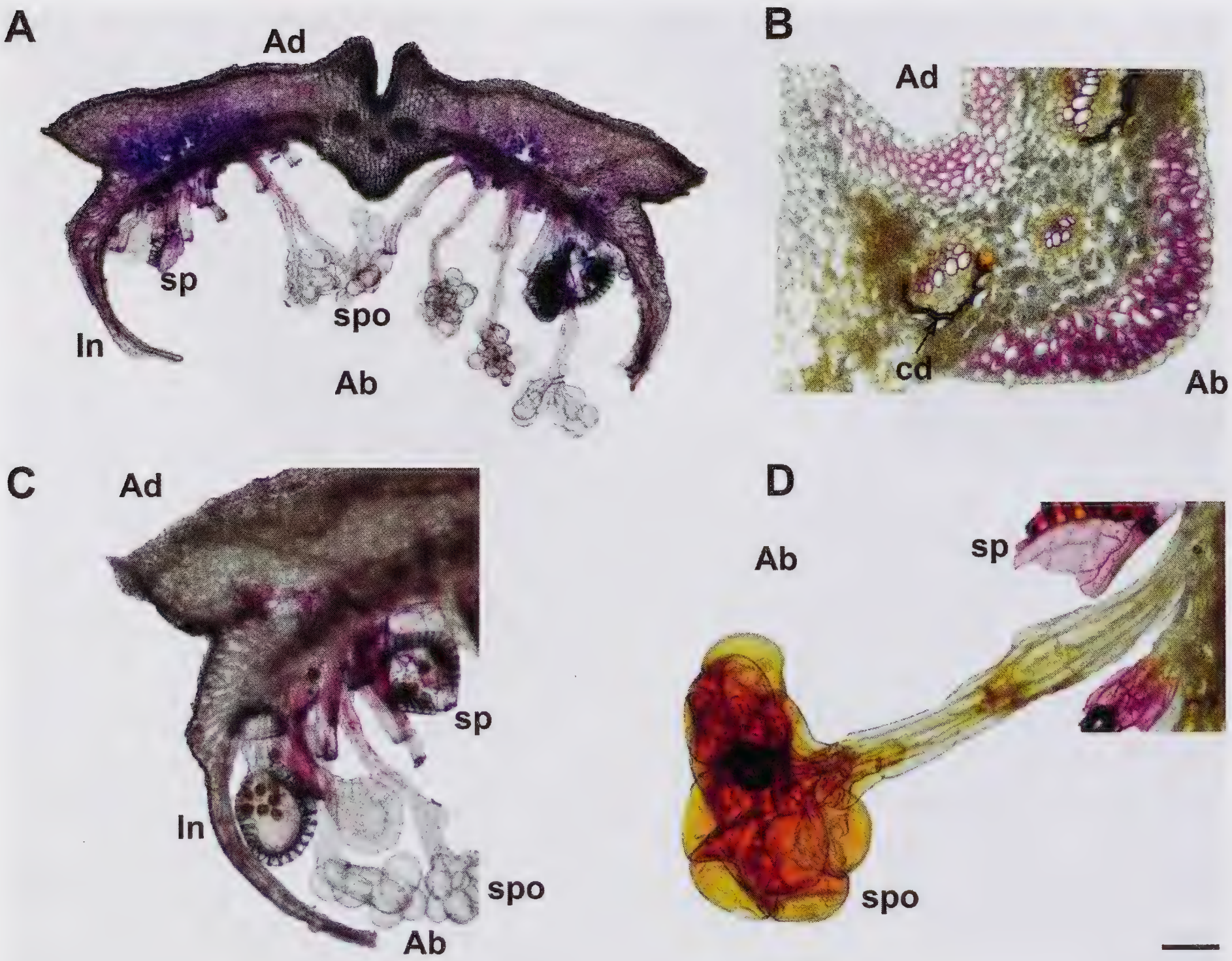

FIG. 3. Cross sections of *P. nesophilum* fertile pinnae (*Sundue 4251*). A. Complete cross section of the fertile pinnae stained with TBO; B. Detail of the costae cross section stained with fluoroglycine; C. Detail of the receptacle cross section stained with fluoroglycine; D. Detail of a sporangiaster. Ab= abaxial; Ad= adaxial; in= indusium; cd= circundermal sheath; sp= sporangia; spo= sporangiasters. Bar = 90 μm in A; 225 μm in B; 130 μm in C; 100 μm in D.

DISCUSSION

This report of sporangiasters in *Parablechnum nesophilum* represents the first recorded occurrence of them in the family. Neither sporangiasters nor any other type of paraphyses have been previously recorded in the Blechnaceae (Rolleri, Prada, and Passarelli, 2008; Rothfels *et al.*, 2012; Prada *et al.*, 2016). The sporangiasters were not previously overlooked because they are hard to observe; we immediately noticed the vitreous spherical heads during field work (Fig. 1A, 1B). In dried specimens, however, they darken and become less conspicuous, which probably accounts for their being previously overlooked.

Sporangiasters have also been reported in *Alsophila* (Janssen and Rakotondrainibe, 2006), *Campyloneurum* (Wagner and Farrar, 1976), *Lecanopteris* (Hennipman, 1986), *Loxogramme* (Price, 1990), *Meniscium* (Fernandes and Salino, 2016), and *Vittaria* (Britton and Taylor, 1902). Within these examples, we note that the homology of the paraphyses to sporangia is not always clear; some of these may in fact be derived from hairs or scales rather than sporangia

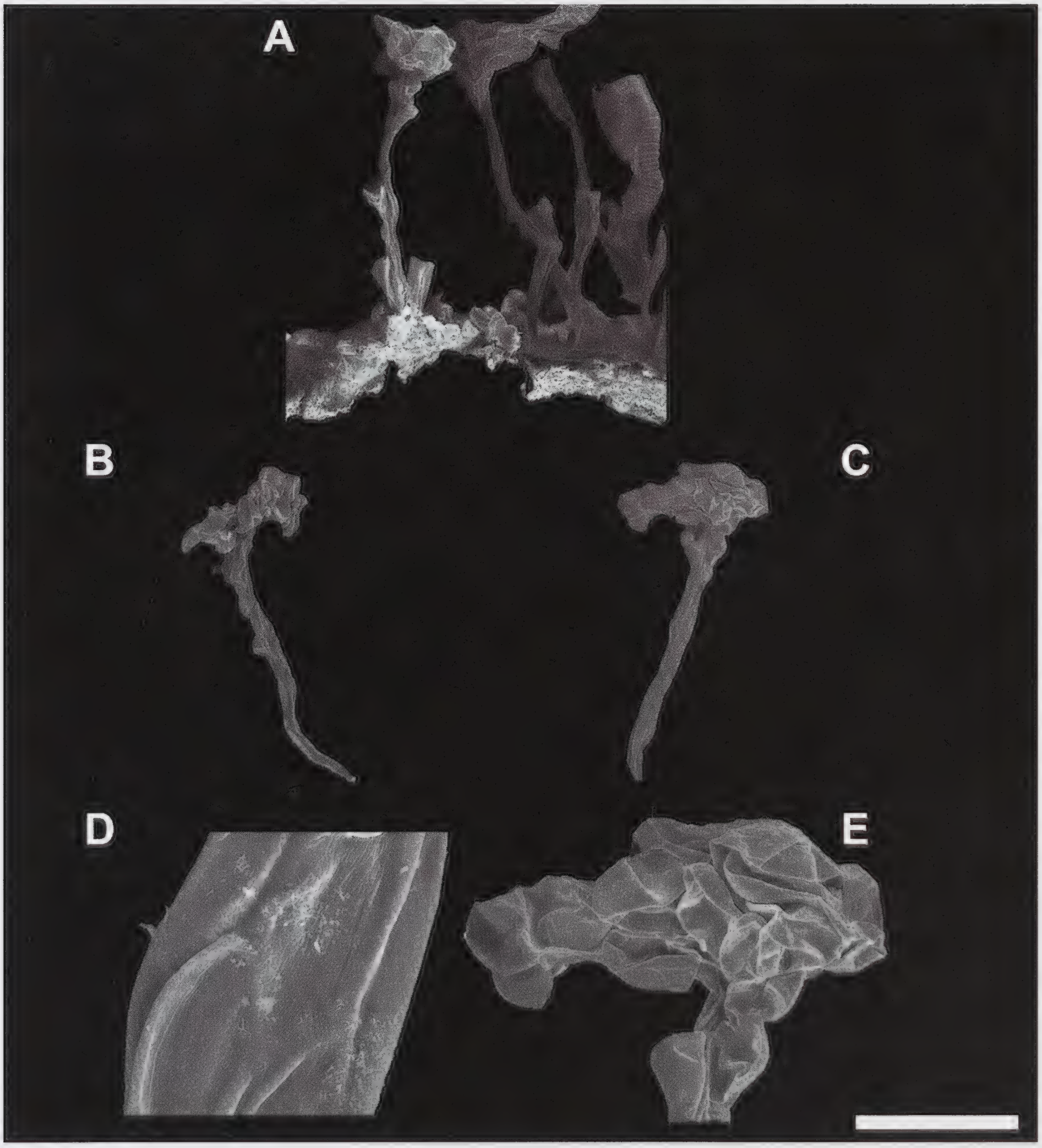

FIG. 4. Sporangiasters of *P. nesophilum* under SEM (*Sundue 4251*). A. Cross section of a fertile pinnae bearing attached sporangiasters; B–C. Sporangiasters; D. Detail of the sporangiaster pedicel; E. Detail of clavate head. scalebar = 530 μm in A–C; 50 μm in E; 200 μm in E.

and would be better described using the general term paraphyses. Others may simply be aborted sporangia that are not otherwise modified. In those cases, an additional term does not seem helpful. Indehiscent sporangia such as those reported by Wang *et al.* (2012) are also different from sporangiasters in that they contain viable spores, but are not otherwise modified. We also acknowledge that some other groups of ferns such as *Acrostichum* (Adams and Tomlinson, 1979) and *Lomagramma* (Moran, Labiak, and Sundue, 2010) have paraphyses that bear a strong resemblance to sporangia; but the term

sporangiaster has never been applied, and more study is needed to establish homology.

As noted by Wagner (1964), paraphyses seem to occur most often in exindusiate ferns. This observation supports the idea that the primary function of paraphyses is to protect developing sporangia. Therefore, they are more likely to evolve in exindusiate plants. With this in mind, the presence of sporangiasters in *P. nesophilum* comes as a surprise, since the species also has a well-developed indusium (Fig. 2). We know very little about the timing of indusial opening in *P. nesophilum*, but it may be that it is early-opening. When *Sundue 4251* was collected, the indusium had already retracted and did not cover the sorus (Figs. 1A, 1B). It seems likely that its sporangiasters act as a second stage of protection for the developing sporangia after the indusium retracts. Alternatively, sporangiasters may influence the vagility of spores by inhibiting dehiscence and limiting the ability of the annulus to fling the spores away from the plant (Wagner *in* Carlquist, 1966). By limiting spore dispersal, the sporangiasters help maximize local recruitment and minimize loss of spores to potentially unfavorable conditions that may occur further away (Carlquist, 1966). The geographic distribution of *P. nesophilum* fits this scenario: it inhabits small islands of eastern Malesia and the western Pacific. Whether sporangiasters have any effect on the vagility of spores in this species remains unknown. Additional anatomical studies of species from the region will hopefully provide more insight.

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Literature Cited

Adams, D. C. and P. B. Tomlinson. 1979. *Acrostichum* in Florida. American Fern Journal 69:42–46.

Baayen, R. P. and E. Hennipman. 1987. Paraphyses of the Polypodiaceae (Filicales). II. Taxonomic part. Beitrage zur Biologie der Pflanzen 62(2): 251-315.

Bower, F. O. 1928. *The Ferns (Filicales). Vol. III. The Leptosporangiate Ferns*. Cambridge University Press.

Britton, E. G. and A. Taylor. 1902. The life history of *Vittaria lineata*. Memoirs of the Torrey Botanical Club 8:185–211.

Cámara-Leret, R., D. G. Frodin, F. Adema, C. Anderson, M. S. Appelhans, G. Argent, S. Arias Guerrero, P. Ashton, W. J. Baker, A. S. Barfod, D. Barrington, R. Borosova, G. L. C. Bramley, *et al.* 2020. New Guinea has the world's richest island flora. Nature 584:579–583.

Carlquist, S. 1966. The biota of long-distance dispersal. III. Loss of dispersibility in the Hawaiian flora. Brittonia 18:310–335.

Chambers, T. C., P. J. Edwards, and R. J. Johns. 2005. *Blechnum puniceum* (Pterophyta: Blechnaceae), a new species from Papua, Indonesia. Contributions to the Flora of Mount Jaya, XVI. Kew Bulletin 60:597–601.

Chen C-W, L. Perrie, D. Glenny, and W.-L. Chiou. 2017. *Sol: Amazing Lycophytes & Ferns of the Solomon Islands*. National Museum of Natural Science, 550 pp.

Copeland, E. B. 1947. *Genera Filicum. The Genera of Ferns*. Chronica Botanica. Waltham.

Fernandes, R. S. and A. Salino. 2016. A new species and a new combination in *Meniscium* (Thelypteridaceae) from Brazil. Phytotaxa 273:175–182.

Hennipman, E. 1986. Notes on the ant-ferns of *Lecanopteris sensu stricto* in Sulawesi, with the description of two new species. Kew Bulletin 41:781–788.

Holttum, R. E. 1991. *Tectaria* group. Flora Malesiana-Series 2, Pteridophyta 2:1–124.

Janssen, T. and F. Rakotondrainibe. 2006. A revision of the fern family Cyatheaceae in the Mascarene Islands. Adansonia 28:213–214.

Lehtonen, S., H. Tuomisto, G. Rouhan, and M. J. Christenhusz. 2010. Phylogenetics and classification of the pantropical fern family Lindsaeaceae. Botanical Journal of the Linnean Society 163:305–359.

Lellinger, D. B. 2002. A modern multilingual glossary of taxonomic pteridology. Pteridologia 3, Washington D.C., USA.

Martens, P. and N. Pirard. 1943. Les organes glanduleux de *Polypodium virginianum* L. II. Structure, origine et signification. Cellule 49:382–400.

Molino, S., C. Prada, J. M. Gabriel y Galán, P. Wasowicz, B. Estébanez, and R. Vázquez. 2020. Sporangia and spores in the fern genera *Spicantopsis* and *Struthiopteris* (Blechnaceae, Polypodiopsdiae). Botanical Review 86:76–92.

Moran, R. C., P. H. Labiak, and M. Sundue. 2010. Phylogeny and character evolution of the bolbitidoid ferns (Dryopteridaceae). International Journal of Plant Sciences 171:547–559.

Parris, B. S. 1997. Receptacular paraphyses in Asian, Australasian and Pacific Islands taxa of Grammitidaceae (Filicales). In: Johns, R.J. (Ed.) *Holttum Memorial Volume*. pp. 81–90. Royal Botanic Gardens, Kew, United Kingdom.

Peterson, R. L. and L. S. Kott. 1974. The sorus of *Polypodium virginianum*: some aspects of the development and structure of paraphyses and sporangia. Canadian Journal of Botany 52:2283–2288.

Prada, C., J. M. Gabriel y Galán, P. Saiz, L. Pasarelli, M. Ciciarelli, and C. Rolleri. 2016. Caracteres diagnósticos de frondas esporógenas y esporangios de *Blechnum* L. (Blechnacceae). Iheringia, Série Botânica 7:161–174.

Price, M. G. 1990. Four new Asian *Loxogramme*. American Fern Journal 80:4–8.

Rolleri, C. H., C. Prada, and L. Passarelli. 2008. Morphological and taxonomical studies in *Blechnum* (Blechnaceae-Pteridophyta): *B. tabulare* and *B. magellanicum*. Anales del Jardín Botánico de Madrid 65:179–195.

Rothfels, C. J., M. A. Sundue, L. Y. Kuo, A. Larsson, M. Kato, E. Schuettpelz, and K. M. Pryer. 2012. A revised family–level classification for eupolypod II ferns (Polypodiidae: Polypodiales). Taxon 61:515–533.

Ruzin, S. E. 1999. *Plant microtechnique and microscopy*. University Press, Oxford, United Kingdom.

Schuettpelz, E., C. W. Chen, M. Kessler, J. B. Pinson, G. Johnson, A. Davila, A. T. Cochran, L. Huiet, and K. M. Pryer. 2016. A revised generic classification of vittarioid ferns (Pteridaceae) based on molecular, micromorphological, and geographic data. Taxon 65:708–722.

Schwartsburd, P. B., L. R. Perrie, P. Brownsey, L. D. Shepherd, H. Shang, D. S. Barrington, and M. A. Sundue. 2020. New insights into the evolution of the fern family Dennstaedtiaceae from an expanded molecular phylogeny and morphological analysis. Molecular Phylogenetics and Evolution 150:106881.

Smith, A. R. 1990. Thelypteridaceae. In *Pteridophytes and gymnosperms* (pp. 263–272). Springer, Berlin, Heidelberg.

Stearn, W.T. 1992. *Botanical Latin. 4th Ed.* Timber Press. Portland.

Sundue, M. A., M. B. Islam, and T. A. Ranker. 2010. Systematics of grammitid ferns (Polypodiaceae): using morphology and plastid sequence data to resolve the circumscriptions of *Melpomene* and the polyphyletic genera *Lellingeria* and *Terpsichore*. Systematic Botany 35:701–715.

Sundue, M.A. and C. J. Rothfels. 2014. Stasis and convergence characterize morphological evolution in eupolypod II ferns. Annals of Botany 113:35–54.

Tryon, A.F. 1965. Trichomes and paraphyses in ferns. Taxon 14:214–218.

Tryon, R. 1965. Paraphyses in the ferns. Taxon 14:213–214.

Wagner Jr, W. H. 1964. Paraphyses: Filicineae. Taxon 13:56–64.

Wagner Jr, W. H. 1965. Fern paraphyses: Comments on recent papers. Taxon 14:299–302.

Wagner Jr, W. H. and D. R. Farrar. 1976. The Central American fern genus *Hyalotricha* and its family relationships. Systematic Botany 1:348–362.

Wei, R., A. Ebihara, Y. M. Zhu, C. F. Zhao, S. Hennequin, and X. C. Zhang. 2018. A total-evidence phylogeny of the lady fern genus *Athyrium* Roth (Athyriaceae) with a new infrageneric classification. Molecular Phylogenetics and Evolution 119: 25–36.

Wilson, K. A. 1959. Sporangia of the fern genera allied with *Polypodium* and *Vittaria*. Contributions from the Gray Herbarium of Harvard University 185:97–127.

Zhang, L. B., S. G. Wu, J. Y. Xiang, F. W. Xing, H. He, F. G. Wang, S. G. Lu, S. Y. Dong, D. S. Barrington, K. Iwatsuki, and M. J. M. Christenhusz. 2013. Pp. 541-725. *Dryopteridaceae. In*: W. Zhengyi, P. H. Raven and H. Deyuan (eds.), *Flora of China, vols. 2–3, Lycopodiaceae through Polypodiaceae*. Missouri Botanical Garden Press. St. Louis.

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At Least Two Evolutionarily Distinct Species within *Histiopteris incisa* Inferred from Molecular and Morphological Evidence

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Abstract.—*Histiopteris incisa* (Dennstaedtiaceae) is one of the most cosmopolitan species among the leptosporangiate ferns. Based on sequences of the chloroplast *rbcL* gene, we recognize two lineages within *H. incisa*, and these differ in scale and leaf morphology. This suggests that at least two evolutionarily distinct species are contained within present circumscriptions of *H. incisa*.

Key Words.—Cosmopolitan fern species, cryptic species, *rbcL*

Accurately delimiting species is important for informed management of biodiversity. In the worst case, endangered species may be overlooked. Without flowers or fruits, ferns generally have fewer conspicuous morphological characters for delimiting species when compared with many flowering plants. This paucity of characters means that many evolutionarily distinct species of ferns may be still undiscovered within presently circumscribed taxonomic species. Indeed, recent molecular analyses have detected multiple cryptic species within morphologically circumscribed species in several fern linages (*e.g.*, Dauphin, Vieu, and Grant, 2014; Masuyama *et al.*, 2002; Murakami *et al.*, 1999; Yatabe *et al.*, 2001, 2009). Evolutionarily distinct species – those lineages that maintain their separate identity from other such lineages and that have their own evolutionary tendencies and historical fate (Simpson, 1961; Wiley, 1978) – can be easier to recognize by molecular markers than morphological characters, because the number of characters from molecular data can be several orders of magnitude greater than those from morphology.

Histiopteris incisa (Thunb.) J. Sm. (Dennstaedtiaceae) is one of the most broadly distributed leptosporangiate fern species in the world. It occurs in Asia, Australasia, Africa, and South America (Brownsey and Perrie, 2018; Ebihara, 2016; Roux, 2009; Yan *et al.*, 2013; Zuloaga, Morrone, and Belgrano, 2008). According to a recent fern classification by PPG1 (2016), approximately

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seven species are recorded in *Histiopteris*. Among them, *H. incisa* is the only species widely distributed in the world (Yan *et al.*, 2013) and the only species recorded in Japan (Ebihara, 2016). Therefore, this species has been recorded in many floras (*e.g.* Horning, 1983; Schwartsburd, 2017) and used for morphological and molecular studies as *the* representative of the genus (Imaichi, 1980; Sun *et al.*, 2018). But what if *H. incisa* as currently circumscribed actually contains several evolutionarily distinct species? Perrie *et al.* (2015) suggested the possibility of several evolutionary entities within *H. incisa* based on molecular data. This idea was reinforced by Schwartsburd *et al.* (2020). In the present study, to identify evolutionary distinct units within *H. incisa*, we collected *H. incisa* in Japan, determined the DNA sequences of the chloroplast *rbcL* gene, and explored morphological differentiation between the DNA lineages.

Materials and Methods

Sampling.—Plants of *Histiopteris incisa* were collected from Yakushima in Kagoshima pref., Tokushima pref., and Nachikatsuura in Wakayama pref. A DNA sample was collected from each plant. Voucher specimens were deposited at the Faculty of Education in Kagawa University. Plant samples analyzed in this study are listed in Table 1.

DNA sequencing.—To extract total DNA, leaf tissue (ca. 10×5 mm^2) was crushed in 2.0 mL microtubes using Beads Crusher μT-12 (TAITEC, Saitama, Japan). After 500 μl of CTAB buffer (100 mM Tris; 1.4 M NaCl; 20 mM EDTA; 2% CTAB) was added to the crushed tissue, the mixture was incubated for 30 min at 50°C. The cloudy suspension was then extracted using an equal volume of chloroform and centrifuged for 5 min at 16,000 $\times$ g. The clear supernatant was transferred to a new microtube. An equal volume of isopropanol was added to the supernatant, and this mixture was centrifuged for 15 min at 16,000 $\times$ g. After washing with 70% ethanol, the precipitated DNA was dissolved in 200 μL of TE buffer (10 mM Tris; 1 mM EDTA, pH 8.0).

The *rbcL* region was amplified from total DNA using the aF and cR primers as described by Hasebe *et al.* (1994). The PCR amplification was conducted using up to 150 ng of total DNA in a 10 μL reaction mixture of 10×PCR buffer, dNTP mixture, 5 pmol of both forward and reverse primers, and 0.25 units of TaKarRa Taq polymerase (TAKARA, Kyoto, Japan). The parameters for PCR were: 95°C for 10 min, followed by 40 cycles at 94°C for 30 s, 50°C for 30, and 72°C for 30 s, followed by a final extension at 72°C for 7 min. We added 5 μL of 0.05× ExoSAP-IT (Thermo Fisher Scientific K. K., Tokyo, Japan) to the PCR products to remove extra oligonucleotides and dNTPs. This was followed by incubation at 37°C for 30 min, and finally the enzymes were deactivated at 80°C for 20 min. The purified PCR products were sequenced using the same primers used for amplification. Direct sequencing was performed using an ABI 3130 automated sequencer (Applied Biosystems, Foster City, California) with BigDye Terminator kit ver. 3.1 (Applied Biosystems) following the manufacturer's protocols.

TABLE 1. Plant materials used in this study (samples analyzed in this study are in Bold) .

Species	Locality	Voucher/Citations	*RbcL* type	GenBank no.
Histiopteris incisa	**Japan, Yakushima, pop. 1**	**Shinohara 2016062301001**	**α**	**LC565608**
		Shinohara 2016062301002	**α**	**LC565609**
		Shinohara 2016062301003	**α**	**LC565610**
		Shinohara 2016062302001	**β**	**LC565615**
		Shinohara 2016062302002	**β**	**LC565616**
		Shinohara 2016062303001	**α**	**LC565611**
	Japan, Yakushima, pop. 2	**Shinohara 2017081201001**	**α**	**LC565599**
		Shinohara 2017081202001	**α**	**LC565600**
		Shinohara 2017081203001	**α**	**LC565601**
		Shinohara 2017081204001	**α**	**LC565602**
	Japan, Yakushima, pop. 3	**Shinohara 20190923001**	**α**	**LC565612**
		Shinohara 20190923002	**β**	**LC565617**
		Shinohara 20190923003	**α**	**LC565613**
		Shinohara 20190923004	**α**	**LC565614**
	Japan, Tokushima	**Shinohara 20170813001**	**α**	**LC565603**
	Japan, Tokushima	**Shinohara 20191010001**	**α**	**LC565604**
	Japan, Tokushima	**Shinohara 20191010002**	**α**	**LC565605**
	Japan, Tokushima	**Shinohara 20191010003**	**α**	**LC565606**
	Japan, Tokushima	**Shinohara 20191010004**	**α**	**LC565607**
	Japan, Wakayama	**Shinohara 20170721001**	**β**	**LC565598**
	New Zealand	Perrie *et al.* (2015)	α	KT983822
	Unknown	Schwartsburd *et al.* (2020)	α	MT416339
	Unknown	Schwartsburd *et al.* (2020)	α	MT416340
	Kenya	Shang *et al.* (2018)	β	MH289644
	China, Guangdong	Morigengaowa *et al.* (2018)	β	MG944781
	Japan, Wakayama	Ebihara, Nitta, and Ito. (2010)	β	AB575780
	French Polynesia, Moorea	Nitta *et al.* (2017)	β	KY099807
	New Caledonia	Perrie, Shepherd, and Brownsey (2015)	β	KT983821
	Fiji	Perrie, Shepherd, and Brownsey (2015)	β*	KT983820
	Unknown	Hasebe *et al.* (1994)	β	U05627
	Unknown	Wolf, Soltis, and Soltis (1994)	β*	U05926
H. sinuata		Perrie, Shepherd, and Brownsey (2015)		KT983823
H. stipulacea		Shang *et al.* (2018)		MH289645
Paesia scaberula		Wolf, Soltis, and Soltis (1994)		U05937
Hiya nigrescens		Shang *et al.* (2018)		MH289643
Hiya distans		Perrie, Shepherd, and Brownsey (2018)		MH918672
Blotiella pubescens		Wolf, Soltis, and Soltis (1994)		U05911

Samples with asterisks are not used in the phylogenetic analysis because of short sequences.

Phylogenetic analyses.—The *rbcL* sequence matrix contained data from 35 accessions: 20 accessions were newly analyzed *H. incisa* for this study; nine accessions were *H. incisa* from previous studies registered in GenBank; plus single accessions each of *H. sinuata* (Brack.) J.Sm. and *H. stipulacea* (Hook.)

Copel. from GenBank; along with *Hiya nigrescens* (Hook.) H.Shang, *Hiya distans* (Hook.) Brownsey & Perrie, *Paesia scaberula* (A.Rich.) Kuhn, and *Blotiella pubescens* (Kaulf.) R.M.Tryon from GenBank as outgroups, following the results of recent phylogenetic studies (Perrie *et al.*, 2018; Schwartsburd *et al.*, 2020; Shang *et al.*, 2018).

The nucleotide sequences were aligned using Clustal W (Thompson, Higgins, and Gibson, 1994) followed by minor manual adjustment using SeqManPro v.8.0.2 (DNASTAR). A maximum-likelihood (ML) phylogeny was inferred using MEGA X (Kumar *et al.*, 2018). For the ML analysis, the Kimura 2 parameter model (Kimura, 1980) was selected under the BICc criterion using MEGA X (Kumar *et al.*, 2018). Node support was assessed by 1000 bootstrap replicates with the same substitution model.

Genetic distances between haplotypes were also calculated using the Kimura 2-parameter model.

Results

Molecular analysis.—The aligned data set of sequences in this study was 1,114-bp of part of the *rbcL* gene with 151 variable and 52 parsimony-informative positions. *Histiopteris* was monophyletic with high bootstrap support (89%; Fig. 1). Plants of *H. incisa* used in this study were resolved into two major clades that formed a polytomy with the clade of *H. sinuata* and *H. stipulacea* (Fig. 1). One clade of *H. incisa* contains plants from Japan (Yakushima and Tokushima) and New Zealand, and the other contains plants from Japan (Yakushima and Wakayama), Kenya, China, French Polynesia, and New Caledonia, referred to here as α and β lineages respectively (Fig. 1). The 20 Japanese samples of *H. incisa* whose DNA lineage was determined in this study are listed in Table 1. The α and β lineages were both widespread, and even occurred sympatrically, in two populations in Yakushima (Table 1). At least 11 base substitutions separate the α and β lineages. The Kimua 2-parameter genetic distances between the two lineages based on *rbcL* sequences ranged from 0.010 to 0.017 substitutions/bp.

Morphological observations.—The two lineages had obvious morphological differences in the scales on their rhizomes (Figs. 2, 3). Plants of lineage *α* exhibited large (4.0–5.0 mm long and 0.5–1.0 mm wide), lanceolate and clathrate rhizome scales, whereas those of lineage *β* have small (2.0–3.0 mm x 0.1–0.2 mm) and linear rhizome scales that are rather hair-like. Furthermore, there was the tendency for the leaf apex of lineage *α* plants to unfurl at more-or-less the same time as their lateral pinnae (Fig. 4A). The lineage *β* plants unfurled their proximal lateral pinnae before the frond apex, which was much delayed (Fig. 4B).

Discussion

The correspondence between the DNA lineages and scale and leaf characteristics is stable even in sympatric populations in Yakushima (Table

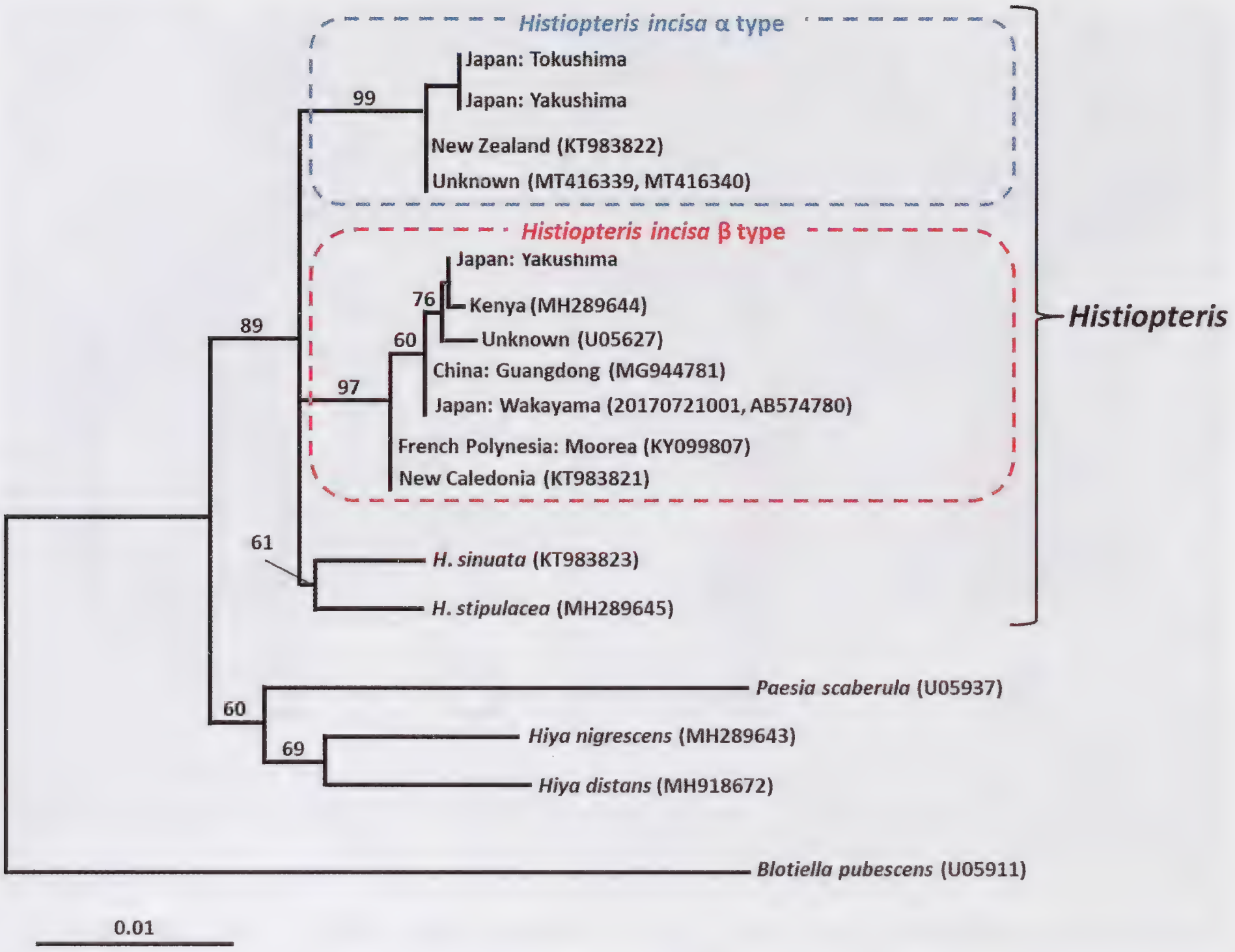

FIG. 1. Phylogenetic tree showing the relationships of *Histiopteris incisa* samples based on the *rbcL* gene inferred from maximum likelihood analysis. Support values are shown above each branch, for branches with >50% maximum likelihood bootstrap support.

1). This concordance suggests that the two DNA lineages are reproductively isolated from each other.

Additionally, relevant sequences from GenBank show that both lineages are distributed outside of Japan (Fig. 1). The *β* lineage is especially widely

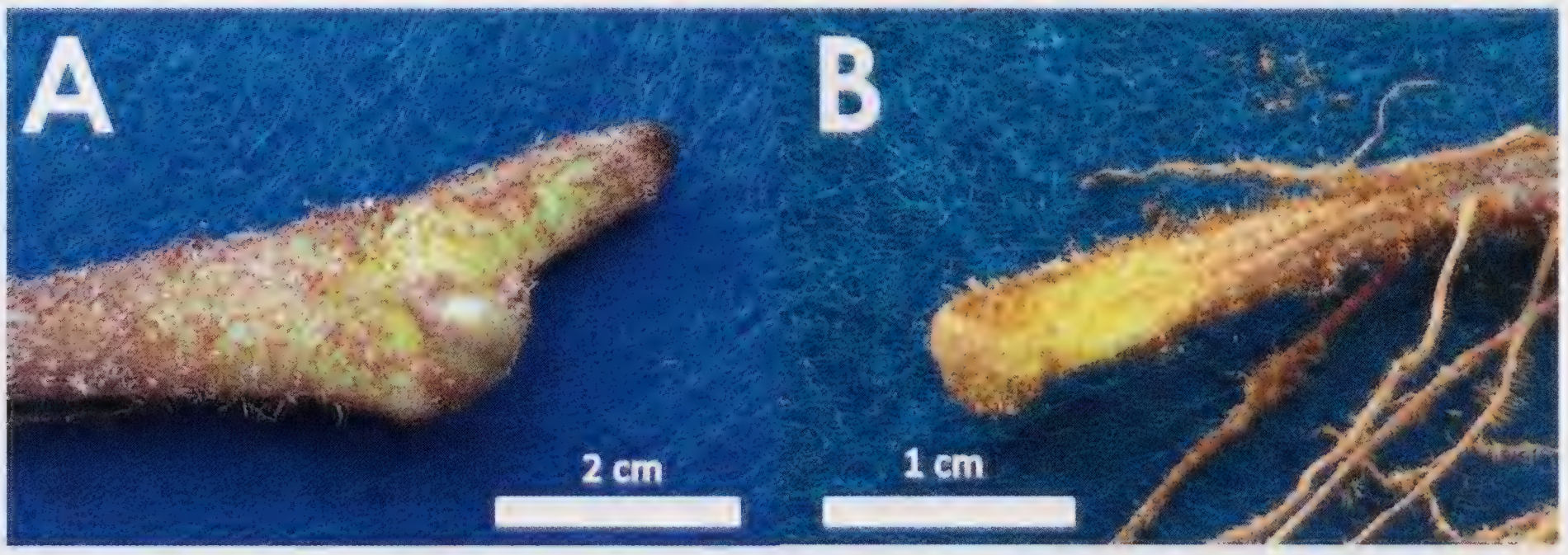

FIG. 2. Rhizomes of the two *rbcL*-based lineages of *Histiopteris incisa*. A: α lineage (Voucher specimen: Shinohara 20190923001). B: β lineage (Voucher specimen: Shinohara 20190923002)

FIG. 3. Scales of the two *rbcL*-based lineages of *Histiopteris incisa*. A, B: α lineage (Voucher specimens: Shinohara20190923001, Shinohara 20191010003).C, D: β lineage (Voucher specimens: Shinohara 20170721001, Shinohara 20190923002).

distributed, including East Asia, Pacific Islands, and Africa. We examined the voucher specimens for the α lineage representative from New Zealand (GenBank KT983822, WELT P027281) and the β lineage representative from New Caledonia (GenBank KT983821, WELT P026234), which confirmed that the correspondence between DNA lineages and scale and leaf morphology also occurs in plants outside of Japan.

Yatabe *et al.* (2009) revealed a correlation between genetic distances among *rbcL* gene sequences and crossing success rates among plants of the same ploidy in the *Asplenium nidus* L. complex (Aspleniaceae) using artificial crossing experiments. Compared to control combinations, crossing success rates decreased between crossing combinations with genetic distances of at least 0.006, and no hybrid individuals were detected if the genetic distances of parent combinations were above 0.013 (Yatabe *et al.*, 2009). In *Ceratopteris thalictroides* (L.) Brongn., plants of the same ploidy with haplotypes differing by only four substitutions in the *rbcL* gene were reproductively isolated from each other (Masuyama *et al.*, 2002). The results of this study show that the α and β lineages of *Histiopteris incisa* differ by at least 11 substitutions, with genetic distances of at least 0.01. These genetic distances suggest that the two lineages, which can occur sympatrically, are likely reproductively isolated. In the present study, the α and β clades of *H. incisa* and a clade of *H. sinuata* and *H. stipulacea* form a polytomy, because there are no synapomorphies for any two of the three clades in the analyzed region. The molecular phylogenetic trees in Perrie *et al.* (2015) and Schwartsburd *et al.* (2020) indicated that *H.*

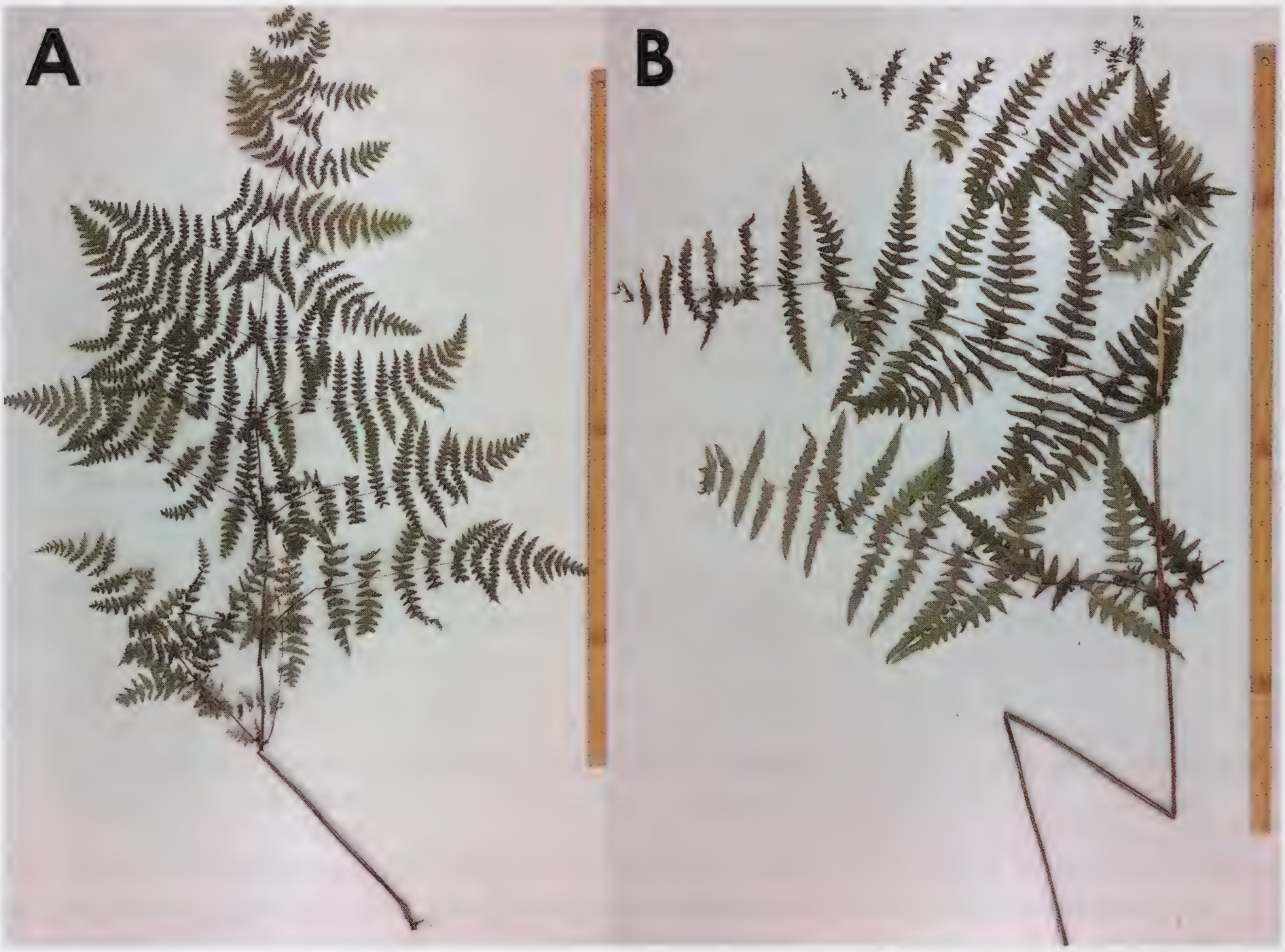

FIG. 4. Leaves of the two *rbcL*-based lineages of *Histiopteris incisa*. A: α lineage (Voucher specimen: Shinohara 20190923001). B: β lineage (Voucher specimen: Shinohara 20190923002). Scale bar = 1 m.

incisa was paraphyletic, with its α clade more closely related to the clade of *H. sinuata* and *H. stipulacea* than to the β clade. Although these studies found little bootstrap support for this relationship, if it is borne out by additional data, it would reinforce the conclusion that the α and β lineages are distinct evolutionary units.

In conclusion, the morphological and molecular data suggest that the α and β lineages of *Histiopteris incisa* are evolutionarily distinct species. We examined the holotype (UPS-THUNB 24932) of *Pteris incisa* Thunb., the basionym of *Histiopteris incisa*, which was collected from South Africa. It contains only the upper portion of a leaf and no rhizome. Evaluating only from the leaf form, the type specimen resembles the α lineage; however, we suspect leaf form may not be an absolutely definitive character, and only the β lineage has been detected from Africa so far (Table 1, Fig. 1). Therefore, we believe it is not yet possible to determine to which lineage the holotype belongs. In the future, we will collect and sequence plants from where the type specimen was collected, to determine which of the lineages should retain the name *H. incisa*.

Tetraploid and diploid plants in *Histiopteris incisa* have been reported from Japan (Kurita, 1972; Mitui, 1973). Therefore, we are also planning to investigate the ploidy levels of both lineages.

Acknowledgements

We are grateful to Dr. Mats Hjertson for sending us images of the type specimen deposited at Uppsala University. We also thank Mr. Kazuhiko Takaie, Mr. Koichi Ohora, Mr. Aiji Narita, and Mr. Syozi Hyodo of the Nippon Fernist Club for collecting samples for this research. We thank the reviewers and editors for their comments that improved the manuscript. This study was financially supported by Grant-in-Aid for Scientific Research (C) No. 15K07188 from Japan Society for the Promotion of Science (JSPS), The 26th Botanical Research Grant from The New Technology Development Foundation, Young Scientists Fund of Kagawa University Research Promotion Program 2020 (KURPP) to WS, and Grant-in-Aid for Scientific Research (B) No. 19H03288 from Japan Society for the Promotion of Science (JSPS) to NM.

Literature Cited

Brownsey, P. J., and L. R. Perrie. 2018. Dennstaedtiaceae. Fascicle 19, *in* I. Breitwieser and A. D. Wilton (eds.), *Flora of New Zealand – Ferns and Lycophytes*. Manaaki Whenua Press, Lincoln.

Dauphin, B., J. Vieu, and R. J. Grant. 2014. Molecular phylogenetics supports widespread cryptic species in moonworts (*Botrychium* s.s., Ophioglossaceae). American Journal of Botany 101:128–140.

Ebihara, A. 2016. *The Standard of Ferns and Lycophytes in Japan 1*. Gakkenn-Plus, Tokyo. (in Japanese)

Ebihara, A., J. H. Nitta, and M. Ito. 2010. Molecular species identification with rich floristic sampling: DNA barcoding the pteridophyte flora of Japan. PLoS ONE 5:e15136. https://doi.org/10.1371/journal.pone.0015136

Hasebe, M., T. Omori, M. Nakazawa, T. Sano, and M. Kato. 1994. *RbcL* gene sequences provide new evidence for the evolutionary lineages of leptosporangiate ferns. Proceedings of the Natural Academy of Sciences USA 91:5730–5734.

Horning, D. S. 1983. A new fern record from the Snares, southern New Zealand. New Zealand Journal of Botany 21:205–208.

Imaichi, R. 1980. Developmental studies on the leaf and the extra-axillary bud of *Histiopteris incisa*. Botanical Magazine, Tokyo 93:25–38.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549.

Kurita, S. 1972. Chromosome numbers of some Japanese ferns (8). Annual report of the Foreign Students College of Chiba University 7:47–53.

Masuyama, S., Y. Yatabe, N. Murakami, and Y. Watano. 2002. Cryptic species in the fern *Ceratopteris thalictroides* (L.) Brongn. (Parkeriaceae). I. Molecular analyses and crossing tests. Journal of Plant Research 115:87–97.

Mitui, K. 1973. A cytological survey on the pteridophytes of the Bonin Islands. The Journal of Japanese Botany 48:247–254.

Morigengaowa, J.-J. Luo, R. Knapp, H.-J. Wei, B.-D. Liu, Y.-H. Yan, and H. Shang. 2018. The identity of *Hypolepis robusta*, as a new synonym of *Hypolepis alpina* (Dennstaedtiaceae), based on morphology and DNA barcoding and the new distribution. PhytoKeys 96:35–45.

Murakami, N., M. Watanabe, J. Yokoyama, Y. Yatabe, H. Iwasaki, and S. Serizawa. 1999. Molecular taxonomic study and revision of the three Japanese species of *Asplenium* sect. *Thamnopteris*. Journal of Plant Research 112:15–25.

Nitta, J. H., J.-Y. Meyer, R. Taputuarai, and C. C. Davis. 2016. Life cycle matters: DNA barcoding reveals contrasting community structure between fern sporophytes and gametophytes. Ecological Monographs 87:278–296.

Perrie, L. R., L. D. Shepherd, and P. J. Brownsey. 2015. An expanded phylogeny of the Dennstaedtiaceae ferns: *Oenotrichia* falls within a non-monophyletic *Dennstaedtia*, and *Saccoloma* is polyphyletic. Australian Systematic Botany 28:256–264.

Perrie, L. R., L. D. Shepherd, and P. J. Brownsey. 2018. *Hiya distans* (Dennstaedtiaceae), a new combination for an Australasian fern previously classified in *Hypolepis*. New Zealand Journal of Botany 56:406–414.

PPG I. 2016. A community-derived classification for extant lycophytes and ferns. Journal of Systematics and Evolution 54:563–603.

Roux, J. P. 2009. Synopsis of the Lycopodiophyta and Pteridophyta of Africa, Madagascar and neighbouring islands. Strelitzia 23:1–296.

Schwartsburd, P. B. 2017. Flora of Espírito Santo: Dennstaedtiaceae. Rodriguésia 68:1559–1575.

Schwartsburd, P. B., L. R. Perrie, P. J. Brownsey, L. D. Shepherd, H. Shang, D. S. Barrington, and M. A. Sundue. 2020. New insights into the evolution of the fern family Dennstaedtiaceae from an expanded molecular phylogeny and morphological analysis. Molecular Phylogenetics and Evolution 150:1–20.

Shang, H, M. Sundue, R. Wei, X.-P. Wei, J.-H. Luo, L. Liu, P. B. Schwartsburd, Y.-H. Yan, and X.-C. Zhang. 2018. *Hiya*: a new genus segregated from *Hypolepis* in the fern family Dennstaedtiaceae based on phylogenetic evidence and character evolution. Molecular Phylogenetics and Evolution 127:449–458.

Simpson, G. G. 1961. *Principles of Animal Taxonomy*. Columbia University Press, New York.

Sun, X., Z. Kang, S. Liu, R. Xu, Z. Wang, T. Wang, and Y. Su. 2018. The complete chloroplast genome sequence of *Histiopteris incisa* (Dennstaedtiaceae). Mitochondrial DNA Part B 3:772–773.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

Wiley, E. O. 1978. The evolutionary species concept reconsidered. Systematic Zoology 27:17–26.

Wolf, P. G., P. S. Soltis, and D. E. Soltis. 1994. Phylogenetic relationships of dennstaedtioid ferns: evidence from *rbcL* sequences. Molecular Phylogenetics and Evolution 3:383–392.

Yan, Y. H., X. P. Qi, W. B. Liao, F. W. Xing, M. Y. Ding, F. G. Wang, X. C. Zhang, Z. H. Wu, S. Serizawa, J. Prado, M. Funston, M. G. Gilbert, and H. P. Nooteboom. 2013. Dennstaedtiaceae. Pp. 147–168, *in* Z. Y. Wu, P. H. Raven and D. Y. Hong (eds.), *Flora of China. Vol. 2–3 (Pteridophytes)*. Science Press, Beijing.

Yatabe, Y, S. Masuyama, D. Darnaedi, and N. Murakami. 2001. Molecular systematics of the *Asplenium nidus* complex from Mt. Halimun National Park, Indonesia; Evidence for reproductive isolation among three sympatric *rbcL* sequence types. American Journal of Botany 88:1517–1522.

Yatabe, Y., W. Shinohara, M. Matsumoto, and N. Murakami. 2009. Patterns of hybrid formation among cryptic species of bird-nest fern, *Asplenium nidus* complex (Aspleniaceae) in West Malesia. Botanical Journal of Linnean Society 160:42–63.

Zuloaga, F. O., O. Morrone, and M. J. Belgrano. 2008. *Catálogo de las Plantas Vasculares del Cono Sur (Argentina, Sur de Brasil, Chile, Paraguay y Uruguay). Volumen 1. Pteridophyta, Gymnospermae, Monocotyledoneae*. Missouri Botanical Garden Press, St. Louis.

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Shorter Note

***Aspidotis densa* (Pteridaceae), A Noteworthy First Record from Alaska.**—*Aspidotis densa* (Brack.) Lellinger was found growing within crevices of a dunite rock formation on Annette Island, one of the most southern islands of the Alaska Alexander Archipelago in Southeast Alaska. This is the first record of the fern from Alaska and represents the most northern collection for this taxon (Figure 1). The taxon ranges from the Pacific Northwest to central California and eastward to western Montana. Scattered occurrences are located in western Wyoming, northern Utah, and southern California, as well as disjunct populations in Quebec (Figure 1).

Aspidotis densa (Brack.) Lellinger. U.S.A., Alaska. Annette Island, Yellow Hill. Approx. 2.75 km south of Metlakatla. 55° 6.128′ N, 131° 34.535′ W. 31 Jul 2019. Between crevices of dunite bedrock. *Kristen Lease s.n.* with *Genelle Winter* (UAAH 13265! & 13266!).

The population was small (exact count not performed) and restricted near the summit (165 m elev.) of Yellow Hill in several microhabitats. *Aspidotis densa* was growing in dense mats in boulder crevices (some vertical) or in very shallow, well-draining soil. Associated species were not recorded, but habitat photos show *A. densa* generally alone or in shallow crevices with *Carex scirpoidea* Michx. The flora of Yellow Hill has not been thoroughly documented, but eleven vascular plant collections were made in 2001 by a regional expert during a very brief site visit. The collectors noted that *A. densa* was easily overlooked and confused with adjacent *Juniperus communis* L. Other pteridophytes from Yellow Hill include *Adiantum aleuticum* (Rupr.) Paris, *Cryptogramma acrostichoides* R. Br., and *Polystichum kruckebergii* W. H. Wagner.

Yellow Hill is a Cretaceous ultra-mafic rock formation of exposed dunite, the only exposed dunite on the island. Dunite can be a precursor form of serpentine material. *Aspidotis densa* is known to be an indicator of serpentine soils (Kruckeberg, International Geology Review 46:857–860. 2004), though not as strongly associated as previously considered. In its western distribution, the taxon can be found either associated or not with serpentine soil, but the disjunct populations occurring in eastern Canada are restricted to serpentine substrates (Harris and Rajakaruna, Northeastern Naturalist 16:111–120. 2009). Other exposed dunite formations or serpentine soils in Southeast Alaska are high priority areas to search for additional populations of the taxon.

The closest vouchered population of *A. densa* is isolated on a mountain 1,000 km east of the Alaska population in central British Columbia, but the main contiguous range from the Pacific Northwest is 1,135 km south, on Vancouver Island, B.C. (Figure 1). Thought to be rare events, pteridophytes are capable of long distance dispersal, confounding the local origins via vicariance or dispersal mechanisms (Wagner, Annals of the Missouri Botanical Garden 59:203–217. 1972; Wolf, Schneider, and Ranker, Journal of Biogeography

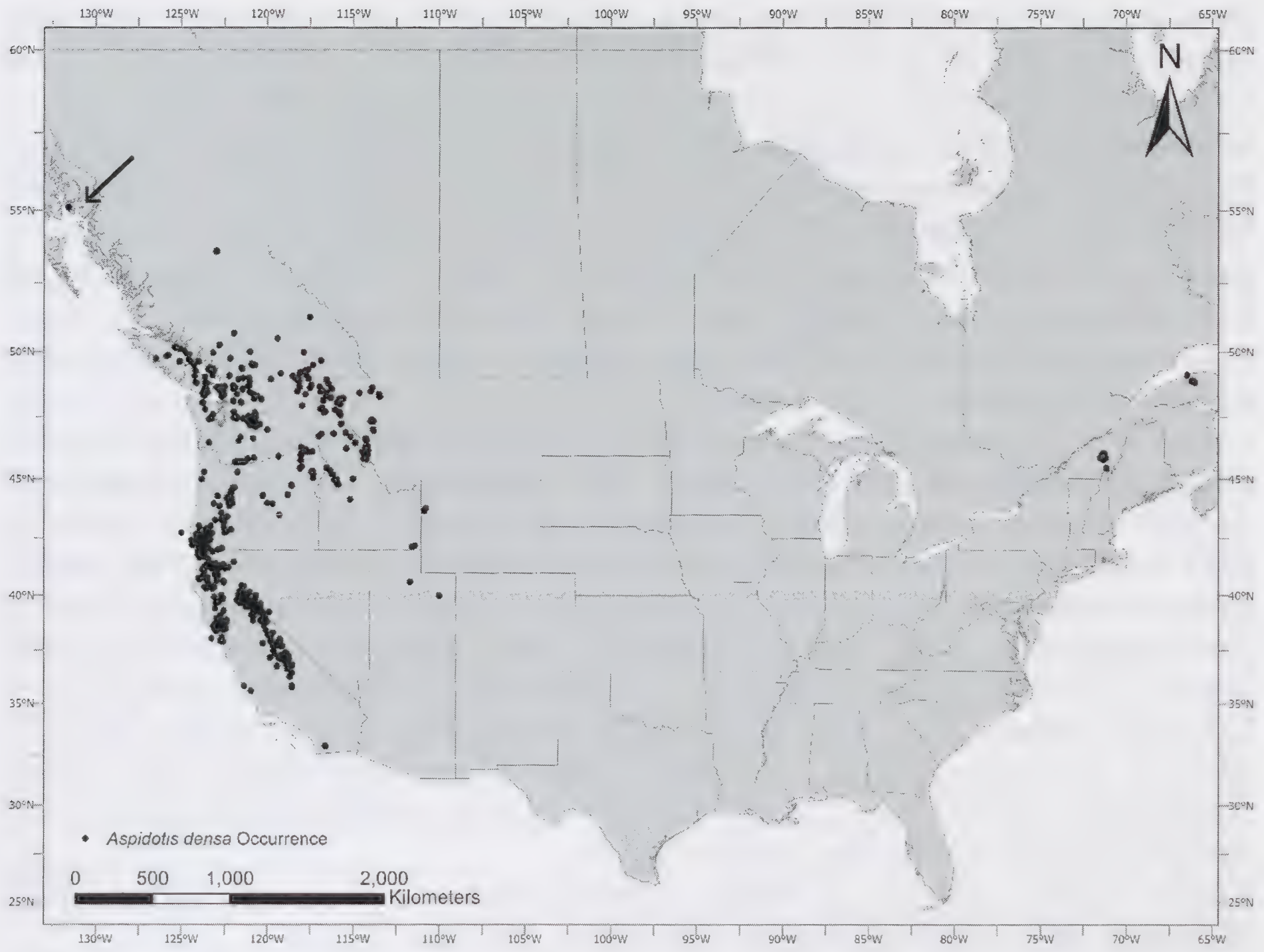

FIG. 1. Occurrences of *Aspidotis densa* originating from herbaria records and observations (Biodiversity occurrence data published by: RENO:V (Accessed through Data Portal, http//:swbiodiversity.org/index.php), November 11, 2020; Consortium of Pacific Northwest Herbaria Specimen Database (CPNWH). 2020. Data provided by: BABY, CIC, EWU, HJAEF, HPSU, HSC, ID, IDS, LINF, MONT, MONTU, NOCA, OSC, PLU, RM, SRP, UAAH, UBC, V, WS, WTU, WWB. https://www.pnwherbaria.org (Accessed May 5, 2020); Wyoming Natural Diversity Database. http://www.uwyo.edu/wyndd/find-data-info/about-our-data-information/codes-and-definitions/species-concern.html (Accessed May 5, 2020); GBIF.org (20 May 2020) GBIF Occurrence Download https://doi.org/10.15468/dl.vgkqxy). Arrow highlights Alaska occurrence of interest.

28:263–270. 2001). Some rare taxa with similar disjunct patterns in Southeast Alaska include *Stachys mexicana* Benth., *Lobelia dortmanna* L., *Cardamine angulata* Hook., *Brasenia schreberi* J.F. Gmel., and *Asplenium trichomanes* L. The Alexander Archipelago complex has long been proposed as a glacial refugium and some evidence supports this hypothesis for plants and animals (*e.g.*, Soltis *et al.*, American Journal of Botany 78:1379–1390. 1991; Sawyer *et al.*, Ecology and Evolution 9:1777–1797. 2019). Given the soil specificity, range from main population, and other regional flora with similar disjunct patterns, vicariance is a reasonable hypothesis but further testing would be needed.

During the curation of the voucher at UAAH and researching *A. densa* distribution, the lead author found the occurrence to be documented two months later on iNaturalist, a popular mobile device application used to document natural history sightings. No voucher was collected, but clear

photos still demonstrated positive identification by the citizen science community, elevating it to "Research Grade" (GBIF.org, (20 May 2020) GBIF Occurrence Download https://doi.org/10.15468/dl.vgkqxy). While there can be concern or pitfalls in accepting citizen science data for scientific research (*e.g.*, Dickinson, Zuckererg, and Bonter, Annual Review of Ecology, Evolution, and Systematics 41:149–172. 2010), this iNaturalist observation would have been the first documentation in Alaska if *Kristen Lease s.n.* had not been collected two months prior. Indeed, this citizen science tool can be helpful in documenting the regional flora, especially in remote and difficult to access locations in Alaska.

The Global Status of *A. densa* is ranked Secure (G5) (NatureServe, https://explorer.natureserve.org/, Accessed: May 5, 2020). The Subnational and Provincial status varies from Secure (S5) in British Columbia, Vulnerable (S3) in Quebec, and Critically Imperiled (S1) in Wyoming and Utah. At the Subnational scale for most of its range in the Pacific Northwest, the taxon is Not Assessed or Under Review (SNR/SU). With this new record, the Alaska Natural Heritage Program assigns a conservation subnational rank of S1—Critically Imperiled in Alaska (AKNHP, Alaska Rare Plant Dataset. 2020).—

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Shorter Note

***Asplenium scolopendrium* var. *americanum* (Aspleniaceae) Found in New Mexico, USA.**—*Asplenium scolopendrium* var. *americanum* (Fernald) Kartesz & Gandhi (the American hart's-tongue fern) is among the most distinctive and rarest members of the North American fern flora. First discovered near Syracuse, New York by Frederick Pursh in 1807, reports of new populations of this taxon have drawn considerable interest, due to its rarity, unusual distribution, and close relation to the well-known European hart's-tongue fern, *A. scolopendrium* var. *scolopendrium* L. Following Pursh's initial discovery, additional populations were found in Tennessee (Williamson, Bulletin of the Torrey Botanical Club 6:347–348. 1879), Ontario (Soper, American Fern Journal 44:129–147. 1954), Alabama (Short, American Fern Journal 69:47–48. 1979), and Michigan (Futuyma, American Fern Journal 70:81–87. 1980). Known localities in the United States, where the species is protected under the Endangered Species Act (ESA), are mostly restricted to areas with heavy lake-effect snow on limestone escarpments in the Great Lakes region, with small disjunct populations in limestone sinkholes and caves in the southeastern US. Plants from Mexico and Haiti have sometimes been treated as a distinct variety (in the segregate genus *Phyllitis* as *P. scolopendrium* var. *lindenii* (Hook.) Fernald) or even a different species (*P. lindenii* (Hook.) Maxon) but little evidence apart from geography has been presented to support their distinctiveness from *A. scolopendrium* var. *americanum*, and they have been treated as synonyms of the latter in recent treatments (e.g., Mickel and Smith, Pteridophytes of Mexico, 2004). Even under this broader taxonomic concept, the American hart's-tongue fern is restricted to a few scattered localities, and no significant range extensions have been reported since the discovery of plants in Nuevo León, Mexico, in 1983 (Arreguín-Sánchez and Aguirre-Claverán, Phytologia 60:399–403. 1986).

In February 2017, two of us (L. Baumann and E. Weaver) found a population of *A. scolopendrium* growing in a protected microsite within a small lava tube in El Malpais National Monument, near Grants, Cibola County, New Mexico. The site was discovered during regular surveys of cave features associated with the McCartys lava flow, which comprises the youngest portion of the Zuni-Bandera volcanic field (Dunbar and Phillips, New Mexico Geology 16: 80. 1994). In February 2020, the five authors coordinated a trip to survey the population and search for additional possible localities. A snowstorm delayed the start of the trip and nearly caused its cancellation, but conditions settled and we were able to get in the field by early afternoon. Even in good weather conditions, finding the *A. scolopendrium* population would be difficult; reaching it involves a 40km drive and hiking nearly 2km across a lava field of

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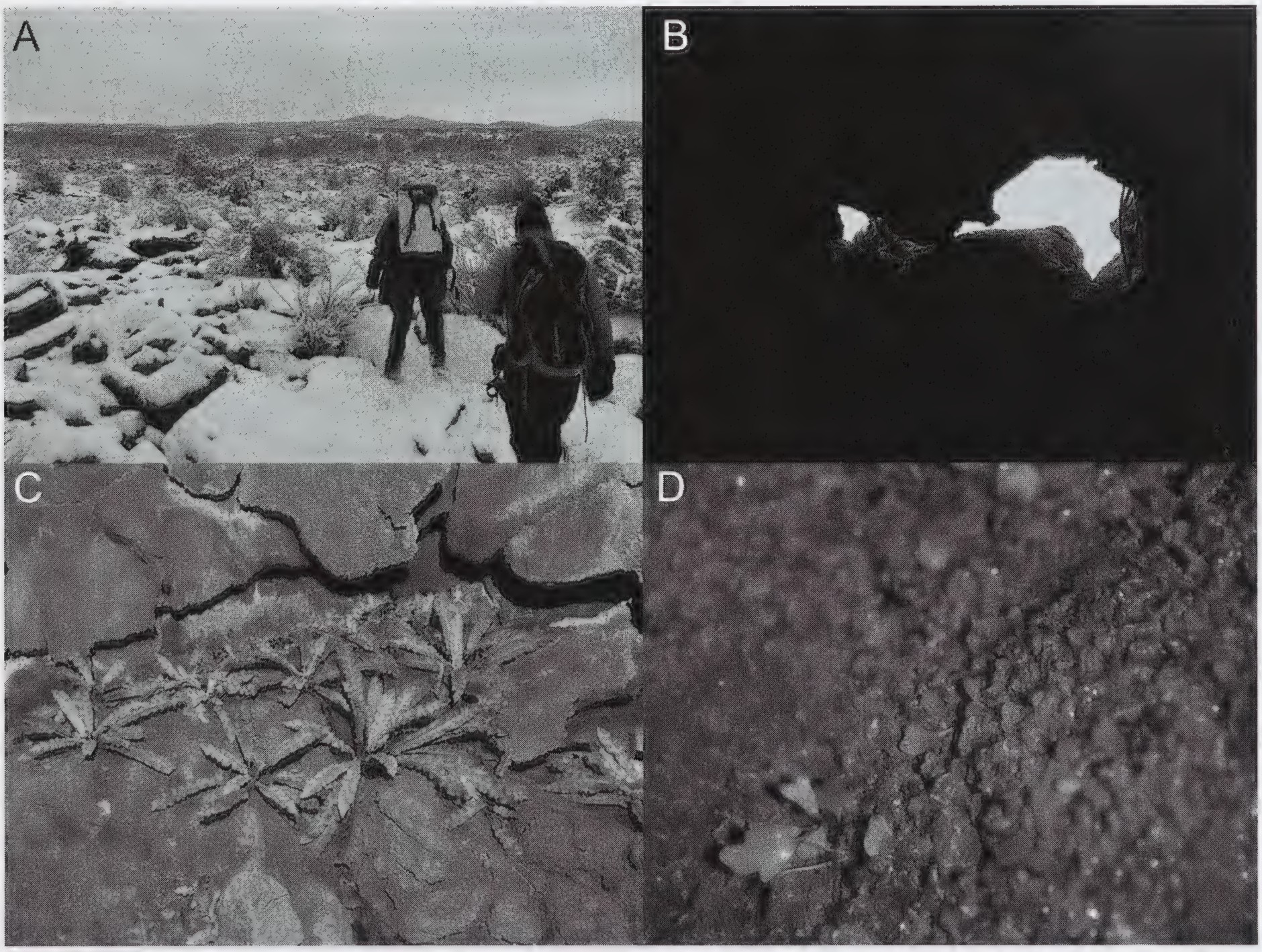

FIG. 1A. Snow-covered lava field near *A. scolopendrium* var. *americanum* site in Cibola County, New Mexico. FIG. 1B. Entrance to lava tube with *A. scolopendrium* var. *americanum* population, viewed from within. FIG. 1C. Mature and immature sporophytes *in situ*. FIG. 1D. Gametophytes and sporelings *in situ*.

sharp and deeply dissected basalt (Fig. 1A). Though covered with a thin layer of snow at the time of our visit, the lava field surrounding the *A. scolopendrium* site is evidently very dry for much of the year, with the local flora being comprising xerophytic species, including: *Opuntia phaeacantha* Engelm., *O. polyacantha* Haw., *Pinus edulis* Engelm., *Cylindropuntia imbricata* (Lam.) F.M. Knuth, *Fallugia paradoxa* (D. Don) Endl. ex Torr., *Atriplex canescens* (Pursh) Nutt., *Yucca baccata* Torr., and *Heterotheca villosa* (Pursh) Shinners.

Given the extreme drought sensitivity of *A. scolopendrium* (Testo and Watkins, American Journal of Botany 100:2261–2270. 2013) and its strong association with limestone elsewhere in its range, its occurrence in such a dry and exposed volcanic landscape may seem incongruous. However, the abundant fissures and lava tubes maintain microclimates with high humidity and cool temperatures year-round (Lindsey, Ecological Monographs 21:227–253. 1951). These microclimates serve as refugia for a number of mesic-adapted plants not found elsewhere in the area, including *A. trichomanes* subsp. *trichomanes* L. (Lindsey, American Fern Journal 35:109–113. 1945) and the

mosses *Sanionia uncinata* (Hedw.) Loeske, *Eurhynchium pulchellum* (Hedw.) Jenn., *Paraleucobryum enerve* (Thed.) Loeske, *Platydictya jungermannioides* (Brid.) H.A. Crum, and *Tortella tortuosa* (Schrad. ex Hedw.) Limpr. (Lindsey, 1951). Ecologically, these habitats are similar to the sinkholes where *A. scolopendrium* var. *americanum* occurs in the southeastern United States and "rockhouse" habitats in the Appalachians, which also harbor unusual floristic assemblages, including members of mostly tropical fern groups, such as *Vittaria* (Farrar and Mickel, American Fern Journal 81:69–75. 1991), *Stegnogramma* (Watkins and Farrar, Brittonia 57:183–201. 2005), *Moranopteris* (Farrar, Science 155:1266–1267. 1967), and several Hymenophyllaceae genera (Pinson *et al.* International Journal of Plant Sciences 178:1–18. 2017).

The lava tube in which the *A. scolopendrium* population occurs is roughly spherical, about 4 m in diameter, with a small opening in the ceiling that is approximately 2.5 feet across (Fig. 1B). We accessed the lava tube with a cable ladder and safety line, as a vertical drop of around 3 m is required to reach the plants on the floor of the lava tube. The air inside the lava tube was very humid and warmer (~3.3°C) than the surface temperature (-4°C). The ferns were growing only on the floor of the lava tube and were most abundant on the south and west sides, presumably where adequate sunlight reaches them. A thin soil layer was present in some areas, but most plants were growing in direct contact with the underlying basalt. A whitish mineral deposit was present on some of the basalt on which the ferns were growing; we believe this to be gypsum and are conducting analyses to characterize it. The population was vigorous and large given the available habitat: we counted six fertile sporophytes and 66 immature sporophytes (longest leaf > 2.5cm) and estimated there were thousands of sporelings (sensu Hunter, American Journal of Botany 9:28–36. 1922; longest leaf < 2.5cm) and many thousand gametophytes (Figs 1C & 1D). No other species of fern were found in the lava tube. We found that the abundant gametophytes had capitate glandular hairs on the margins of the thalli and many possessed proliferations like those reported in lab-cultured gametophytes of *A. scolopendrium* var. *americanum* grown from spores collected from plants in New York (Testo and Watkins, Bulletin of the Torrey Botanical Society 138:400–408. 2011).

Based on our current understanding of the morphological characteristics of the gametophyte, the low probability that the population is introduced, and our current understanding of the distribution of a *A. scolopendrium*, we conclude that the population is in fact *A. scolopendrium* var. *americanum*. Mature sporophytes observed generally appear most similar (in terms of indument and leaf shape) to plants from populations from Tennessee and Alabama, USA; it is unclear at this point if these similarities reflect affinities of these populations or the fact that both occur in cave-like habitats. Interestingly, many of the mature sporophytes possessed at least some leaves with deeply hastate lamina bases, which we have observed sporadically in some specimens of *A. scolopendrium* var. *scolopendrium* but never in *A. scolopendrium* var. *americanum*. Although unusual, we do not believe this similarity reflects a

close relationship with Eurasian plants, especially given the rare and sporadic appearance of this morphology in the latter.

Given the difficulty in separating infraspecific taxa of *Asplenium scolopendrium* and the uncertainty surrounding the evolutionary history of the group, additional study of the New Mexican populations in a broader context is badly needed. The discovery of this population, which is over 1200 km away from the nearest known locality in Nuevo León, Mexico, and 2500 km away from the core populations of *A. scolopendrium* var. *americanum* in the Great Lakes region of the United States and Canada, adds an additional layer of complexity to our understanding of this species. In addition, though weather conditions during our expedition prevented the exploration of other sites, earlier surveys suggest that at least one additional population occurs in the area. Establishing the taxonomic affinities of these newfound populations is a priority for future research, and we collected several leaves as vouchers (*W. L. Testo 2431*, NY, UNM, US) and additional leaf fragments for future genomic analyses, under permit ELMA-2020-SCI-0003.

Given the occurrence of this population in an isolated area with minimal human traffic, it is unlikely to be disturbed by human activity. The population is demographically balanced and the plants we observed were healthy, with the only damage observed being several leaves scattered on the floor of the lava tube, presumably as a result of herbivory, perhaps by packrats (*Neotoma* spp.). Finally, the population's occurrence within a national monument provides it additional protection and monitoring by the National Park Service. The U.S. Fish and Wildlife Service has initiated a 5-Year Review (89 FR 39113) of the federal listing status of *A. scolopendrium* var. *americanum* in accordance with Section 4(c)(2) of the ESA, and this new population and range extension will be included in this review.

This discovery of *Asplenium scolopendrium* in a xeric lava field in New Mexico also adds to the list of fern species with disjunct populations in the southwestern United States and adjacent Mexico. Rock-dwelling species of *Asplenium* are prominent on this list; along with *A. scolopendrium*, *A. adiantum-nigrum* L., *A. dalhousiae* Hook., *A. exiguum* Bedd., *A. monanthes* L., *A. platyneuron* (L.) Britton, Sterns, & Poggenb., and *A. trichomanes* all are represented in this region by populations well outside of their core ranges (Wagner et al., Aspleniaceae, in: Flora of North America Editorial Committee (eds.), The Flora of North America, Vol. 2. Oxford University Press, New York, NY, pp. 228–245. 1993). Some of these disjunctions are truly remarkable: apart from occurrences in the southwestern United States and northern Mexico, *A. dalhousiae* and *A. exiguum* are restricted to eastern Asia, and *A. adiantum-nigrum* is otherwise almost entirely a Eurasian species.The occurrence of these taxa thousands of kilometers outside of their contiguous ranges attest both to the capacity of fern spores for long-distance dispersal and to the potential of climatically buffered microsites—particularly those associated with rock formations—to harbor unusual biota (Smith, MacNeill, and Richard, Madroño 40:174–176. 1993; Farrar, Journal of the Torrey Botanical Society 125:91–108. 1998).

The existence of such isolated populations of *A. scolopendrium* and other mesophytic fern taxa in xeric regions of western North America also raises questions about how they successfully established. Given the long distances involved in any dispersal scenario, it is most probable that these populations arose following colonization events involving a single spore. Of the *Asplenium* species discussed above, only *A. monanthes* is known to be apomictic (Smith and Mickel, Brittonia 29:391–398. 1977), suggesting that initial establishment of populations of the remaining species involved intragametophytic selfing (gametophyte selfing). Although frequently invoked in discussions of fern biogeography (Barrington, Journal of Biogeography 20:275–279. 1993), this reproductive strategy appears to be uncommon in most fern lineages (Soltis and Soltis, American Fern Journal 80:161–172. 1990; Haufler *et al.*, BioScience 66:928–937. 2016) and lab experiments suggest that *A. scolopendrium* var. *americanum* is an obligate outcrosser (Testo and Watkins, 2011). Although this observation on the taxon's reproductive biology is seemingly incongruous with the existence of disjunct populations like the one reported here, there are several possible explanations. First, it is possible, if unlikely, that the population's founder event involved multiple spores that dispersed as a unit (Kramer, Gardeners' Bulletin, Singapore 30:79–83. 1977), or even a whole sporangium (Gastony, American Journal of Botany 61: 672–680. 1974). Alternatively, the proliferations observed on gametophytes in the newly discovered population may have allowed for the establishment of a multi-gametophyte colony via vegetative reproduction, with subsequent intergametophytic mating. Similar scenarios have been posited to explain patterns of long-distance dispersal in tropical epiphytic ferns (Dassler and Farrar, Brittonia 53:352–369. 2001). A third possibility is simply that there is population-level variation in selfing capacity in *A. scolopendrium* var. *americanum*, and the New Mexico population did in fact establish through intragametophytic selfing. Intraspecific variation in mating systems is known to occur in ferns (Sessa, Testo, and Watkins, New Phytologist 211:1108–1119. 2016), including marked differences between American and Eurasian varieties of *A. scolopendrium*. More detailed study of the reproductive biology of this taxon with a focus on the recently discovered New Mexican population should help address these questions.

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COVER CAPTION: Lellingeria cantarensis. Sendero El Cantar, Los Altos de Cerro Azul, Panama Province, Panamá. ~800 m. Photo credit: Jerry & Linda Harrison